Competent Authority Report

Programme for Inclusion of Active Substances in Annex I to Council Directive 98/8/EC

Amines, N-C10–C16-alkyltrimethylenedi-, reaction products with chloroacetic acid; Ampholyt (PT 2, 3, 4)

CAS-No. 139734-65-9

DOCUMENT IIIA (A7)

Evaluation Report

Rapporteur: Ireland

April 2015

Ampholyt (PT2, 3, 4)

Document A7

CONTENTS

Section A7.1.1.1.1 **Hydrolysis as a function of pH and identification of breakdown products**
Annex Point IIA 7.6.2.1

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Reference

Reference	**Cross-reference A3.5/01:** [redacted] Determination of physico-chemical properties of Tego 2000. Infracor GmbH, Marl, Germany, Report No. AN-ASB 0198, April 16, 2002 (unpublished).
Data protection	Yes
Data owner	Goldschmidt GmbH
Companies with letter of access	No
Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

Guidelines and Quality Assurance

Guideline study	Yes EC method C.7 (92/69/EEC)
GLP	Yes
Deviations	Yes Only a preliminary test was performed. The concentration was not quantified.

Materials and Methods

Test material	As given in Section A2. Ampholyt 20 is a synonym for “TEGO 2000”, obtained as a “product by process”, i.e., a 20% aqueous solution of the pure active.
Lot/Batch number	17EM17
Specification	As given in Section A2, 20% aqueous solution (“product by process”).
Purity	20% of the pure active in water
Further relevant properties	The active substance, obtained as a “product by process”, consists of several chemical species which may not be expected to be uniform regarding their adsorptive properties.
Reference substance	No
Initial concentration of reference substance	Not applicable
Test solution	Data on the test solutions are given in Table A7.1.1.1.1–1.

Section A7.1.1.1.1 Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA 7.6.2.1

Testing procedure		
Test system	See Table A7.1.1.1.1- 2	
Temperature	50 ± 5 °C	
pH	pH 4.0: pH 4.2 to 3.9 was measured during test pH 7.0: n.d. pH 9.0: pH 9.0 to 9.1 was measured during test	
Duration of the test	120 hours	
Number of replicates	Two samples at each observation point.	
Sampling	pH 4: 0, 2.4 and 120 hours after test initiation. pH 7: None (due to a milky white precipitate at pH 7.0 the preliminary test and further testing was not performed) pH 9: 0, 2.4 and 120 hours after test initiation. Analysis was performed immediately after taking the samples.	X1
Analytical parameter	The analysis of the composition of the test item Ampholyt 20 could not be performed since a validated method was not available at the time of testing. Therefore, resolution of single substances and integration of the peaks obtained by HPLC measurement was of poor reproducibility. Nevertheless, the shape of the compared chromatograms of the test item at 0 h reaction time and after 5 h did not show large differences. It was therefore concluded that the probability of hydrolysis (which would have become apparent by new peaks in the chromatogram due to degradation products) is low.	
Preliminary test	Yes The test item (50 ml) was dissolved in 450 ml of buffer as given in **Fehler! Verweisquelle konnte nicht gefunden werden.** below.	

Results

Concentration and hydrolysis values	The composition of the test item Ampholyt 20 could not be analysed since a validated method was not available at the time of testing. Therefore, decrease of test substance concentrations was determined by integration of the peaks obtained by HPLC measurement and given in Table A7.1.1.1.1- 3. A complete dissolution in buffer pH 7.0 could not be achieved: A milky white precipitate was found on the bottom of the reaction flask. Therefore, a preliminary test at pH 7.0 was not carried out. After the preliminary test, further testing at pH 4.0 was not performed due to precipitates in the test solutions. Additional testing of hydrolysis at pH 9.0 was not performed after the preliminary test due to more or less equal chromatograms obtained after 120 h, indicating stability of the test item, and further because no analytical method was validated at the time of testing.	
Hydrolysis rate constante (k_h)	Not applicable as specified above (3.4.7, 4.1)	
Dissipation time	Not applicable.	
Concentration-time data	Not applicable.	

Section A7.1.1.1.1 Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA 7.6.2.1

Specification of the transformation product	The deviation of the values of the area determined by HPLC appeared to be more or less in the same order of magnitude at the beginning of the test and after 120 h (pH 9.0, 50 °C), indicating that at pH 9.0 hydrolysis of the test item was not measurable using the analytical method available at the time of testing.

Applicant's Summary and conclusion

Materials and methods	Tego 2000 was mixed with standard buffer (pH4.0, 7.0, or 9.0), stored protected from light at 50 °C for 120 hours. Samples were analysed using HPLC analysis for determination of differences in shape of chromatograms and area under peaks for indication of hydrolysis of the test item.
Results and discussion	Integration of the peaks was not reproducible at the preliminary test at pH 4.0. The preliminary test at pH 7.0 was not performed due to formation of milky turbidity. At pH 9.0, however, comparison of the HPLC-chromatograms obtained in the preliminary test indicate hydrolytic stability of the test item. Further analytical determination of concentration was not performed due to missing analytical methods at the time of testing.
k_H	Not determined
DT_{50}	Not applicable (hydrolytically stable at pH 9.0 or not to be determined at pH 7.0, 4.0 respectively)
r^2	Not applicable (hydrolytically stable at pH 9.0 or not to be determined at pH 7.0, 4.0 respectively)
Conclusion	The validity criteria can not be considered as fulfilled. However, concerning the hydrolysis of the a.s. the findings obtained by HPLC indicate stability (pH 9.0).
Reliability	3
Deficiencies	Yes A validated analytical method was not available. In addition, the solubility of the test item in the buffer solution (pH 4.0 and 7.0) was not optimal: a milky white turbidity was observed after mixing of the test item with the buffer. Therefore, no reliable conclusions of hydrolysis at pH 4.0 and 7.0 can be drawn.

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	25/01/13
Materials and Methods	Applicant's version is considered acceptable with the following additions: X1 A milky white haze could be seen after dissolution of the test item at pH 4 and throughout the HPLC analysis. This suggests the solubility of the test item in the buffer is not optimal. At pH 7 complete dissolution could not be achieved a milky white precipitate was found on the bottom of the reaction flask. Because of the low sensitivity of the analytical method for the test item in buffer solutions a lower concentration test mixture was not applicable. Consequently, a pretest at pH 7 was not carried out. It is unclear from the study report if the samples were sterilised and if sterility was maintained. According to **Table A7.1.1.1.1- 1** C_0 = 20.83 mg/L at pH 4.0, pH 7, and pH 9, respectively. However, 50 mL TEGO 2000 (20.83 g/100 mL) were dissolved in 450 mL of standard buffer solution. This suggests the initial concentration is 20.83 g/L. The applied concentration does not exceed the reported water solubility (≥ 200 g /L). Percentage recovery is not reported.
Results and discussion	Applicant's version is considered acceptable with the following additions: HPLC chromatograms taken from the study report are presented in **Figure CA7.1.1.1.1-1** and **Figure CA7.1.1.1.1-1**
Conclusion	The solubility of the test item in the buffer solution (pH 4.0 and 7.0) was not optimal: a milky white turbidity was observed after mixing of the test item with the buffer. Therefore, no reliable conclusions can be made in relation to hydrolysis at pH 4.0 and 7.0. The HPLC chromatograms suggests the the mixture is stable at pH 9.0.
Reliability	3.
Acceptability	The results at pH 4 and 7.0 are deemed unacceptable for risk assessment. The pH 9 results are supportive of those observed in the key study (IIIA 7.1.1.1.1-02).
Remarks	This study is used as supportive data only
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.1.1.1.1- 1: Type and composition of buffer solutions (specify kind of water if necessary).

Criteria	Details
Purity of water	Distilled, Milli-Q-water
Preparation of test medium	Aqueous standard buffers: pH 4: citric acid – sodium hydroxide – hydrochloric acid pH 7: potassium dihydrogen phosphate – sodium hydroxide pH 9: boric acid – potassium chloride – sodium hydroxide
Test concentrations [mg/l]	c_0 = 20.83 mg/l at pH 4.0, pH 7, and pH 9, respectively (50 ml TEGO 2000 (20.83 g/100ml) were dissolved in 450 ml of standard buffer solution). The reaction flasks were protected from light.
Temperature [°C]	50 ± 0.5 °C
Controls	None
Identity and concentration of co-solvent	No additional solvents (the test item is already dissolved)
Replicates	Duplicate sampling at any observation point

Table A7.1.1.1.1- 2: Description of test system.

Glassware	General laboratory glassware and equipment
Other equipment	Chromatographic equipment (HPLC pump SP 8810, 8800, Spectra-Physics Inc. variable wavelength monitor, Knauer Inc. Analytical balance (accuracy 0.1 mg) Water bath Temperature sensor Testo 701 pH meter Metrohm E 561
Method of sterilization	Not stated (only preliminary test)

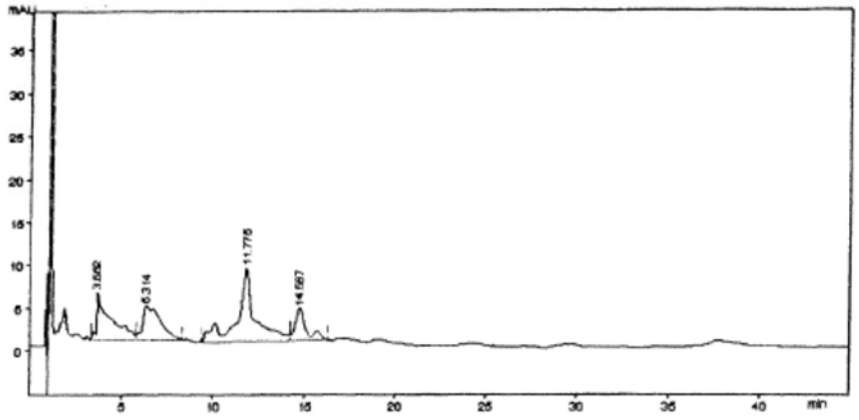
Table A7.1.1.1.1- 3: Hydrolysis of test compound, transformation products and reference substance, expressed as percentage of initial concentrations, at pH 4, pH 7 and pH 9.

Compound	**Sampling times (hours)**					
	0	**0**	**2.4**	**2.4**	**120**	**120**
pH 4 (measured 4.2–3.9)						
Parent compound (Area determined by HPLC and divided by the area of the standard solution	0.64	0.50	0.45	0.44	0.78	0.70
Degree of hydrolysis in (%)	– 12	12	21	22	-37	-23
pH 7						
Parent compound (Area determined by HPLC and divided by the area of the standard solution	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Degree of hydrolysis in (%)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
pH 9 (measured 9.0–9.1)						
Parent compound (Area determined by HPLC and divided by the area of the standard solution	1.05	0.87	0.86	0.77	0.95	0.95
Degree of hydrolysis in (%)	– 9	9	10	20	1	1

n.d. = not determined

CA figures taken from the study report

Chromatogram of the test item diluted 1 : 20 (v : v) with water



Chromatogram of a sample solution (pH 4; 120 hours) with 50 mL/ 450 mL of the test item in buffer sol&ion diluted 1 : 2 (v : v) with water

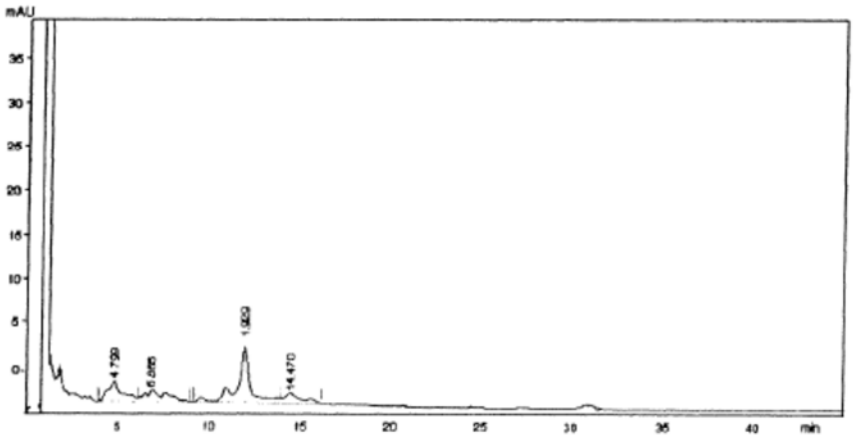
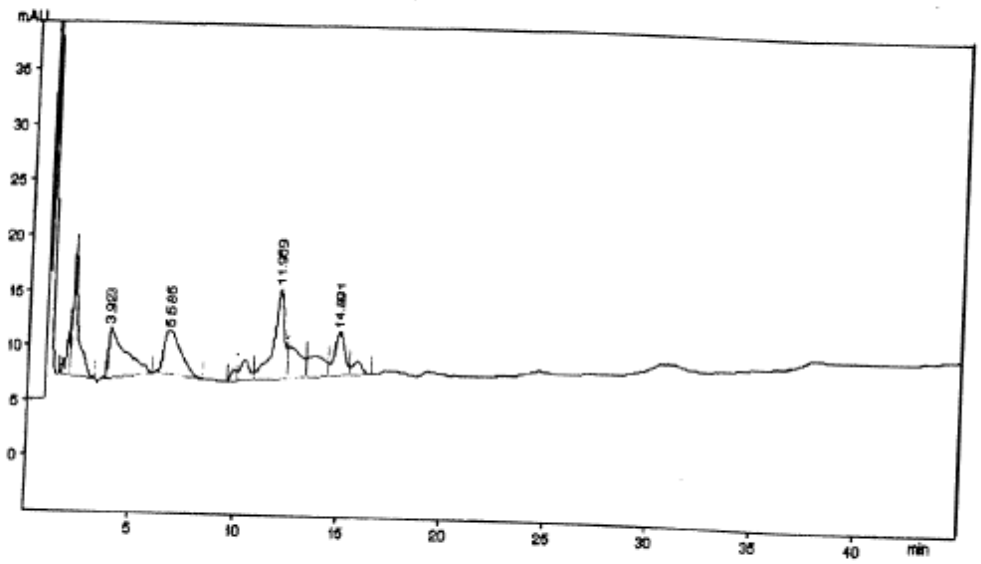


Figure CA7.1.1.1.1-1. HPLC chromatograms

Chromatogram of the test item diluted 1 : 20 (v : v) with pH 9 buffer solution



Chromatogram of a sample solution (pH 9; 120 hours) with 50 mL/ 450 mL of the test item in buffer sol&ion

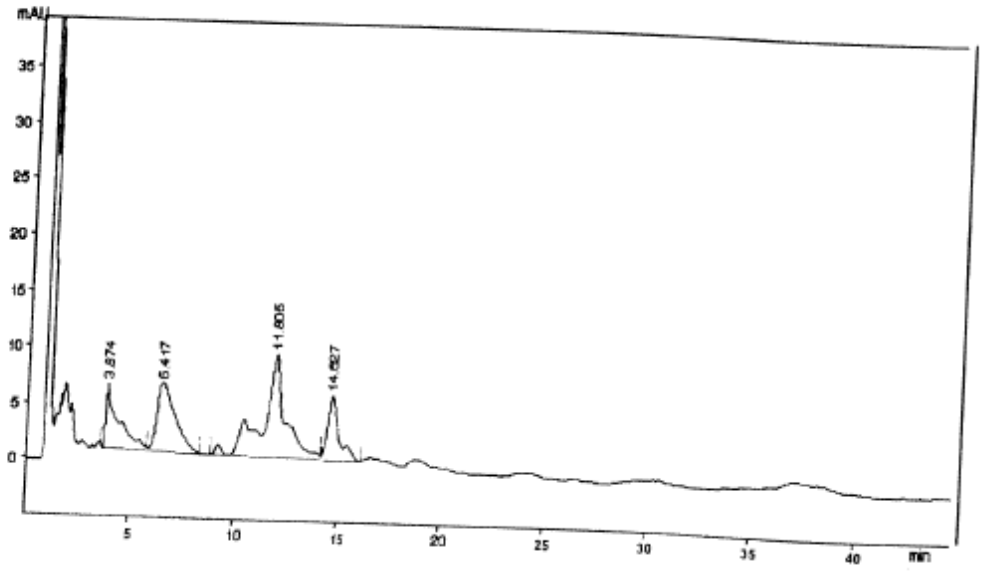


Figure CA7.1.1.1.1-2. HPLC chromatograms from study report

Section A7.1.1.1.1 Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA 7.6.2.1

– Supportive data –

Even though the following reference does not specifically contain additional information about hydrolysis of Ampholyt 20 as a function of pH, it is summarised here and presented in tabular format as supportive data, for the sake of completeness:

Reference	Title	Method	Results
A7.1.1.1.1/01: Brekelmans, MJC (2001): Statement on the determination of the hydrolysis of TEGO 2000 as a function of pH. NOTOX B.B. 's-Hertogenbosch, The Netherlands, unpublished report no.314652, March 05, 2000.	Statement on the determination of the hydrolysis of TEGO 2000 as a function of pH	–	None, the hydrolysis as function of pH could not be analysed due to the lack of a specific analytical method for the test substance at the time of testing.

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	25/01/13	
Materials and Methods	Applicant's version is considered acceptable.	
Results and discussion	Applicant's version is considered acceptable.	
Conclusion	Applicant's version is considered acceptable.	
Reliability	3.	
Acceptability	This data is not suitable for risk assessment	
Remarks		
	COMMENTS FROM ...	
Date		
Materials and Methods		
Results and discussion		
Conclusion		
Reliability		
Acceptability		
Remarks		

Section A7.1.1.1.1 **Hydrolysis as a function of pH and identification of breakdown products**
Annex Point IIA 7.6.2.1

Official use only

Reference

Reference	**A7.1.1.1.1/02:** [redacted] Hydrolysis of Ampholyt 20/100 in water according to the OECD-Guideline 111 "Hydrolysis as a function of pH". Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Schmallenberg, Germany, Report No. EBR-013/7-28, September 09, 2008 (unpublished).
Data protection	Yes
Data owner	Goldschmidt GmbH
Companies with letter of access	No
Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

Guidelines and Quality Assurance

Guideline study	Yes OECD guideline 111 (2002)
GLP	Yes
Deviations	No

Materials and Methods

Test material	Dodecyl-1-^{14}C-labelled Ampholyt 20/100
Lot/Batch number	XVI/38
Specification	Ampholyt 20 is a reaction product (obtained as a "product by process" in form of a 20 % aqueous solution) of alkyl-oligoaamines with chloroacetic acid, the spectrum of alkyl chain lengths ranging from C_{10} to C_{16}. The dodecyl group (C_{12}) dominates the chain length spectrum by approx. 75 %. Ampholyt 20/100 is obtained from Ampholyt 20 by lyophilisation exclusively for testing purposes, thus simply constituting anhydrous Ampholyt 20. The radiolabelled material used for this study, dodecyl-1-^{14}C-Ampholyt 20/100, was deliberately restricted to the C_{12} moiety only, which is considered as representative for the complex active substance. Satisfactory compliance of the labelled substance's composition with that of technical active substance was confirmed by TLC.
Purity	Not defined (mixture of amines, dodecyltrimethylenedi-, reaction products with chloro-acetic acid); however, the signals (by radio-TLC) attributable to the dodecyl-components of Ampholyt 20 account for 94.86% of the radioactivity of the test item.

Section A7.1.1.1.1 Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA 7.6.2.1

Further relevant properties	The a.s. is a multi-component substance as specified in Section A2.
Reference substance	–
Initial concentration of reference substance	–
Test solution	Data on the test solutions are given in Table A7.1.1.1.1- 4 and Table A7.1.1.1.1- 5.
Testing procedure	
Test system	Please refer to Table A7.1.1.1.1- 6. The tubes were incubated for 5 days in darkness in a thermostatically controlled bath.
Temperature	50 ± 0.1 °C
pH	4, 7, and 9 (measured: 4.09, 7.05, and 9.16)
Duration of the test	5 days
Number of replicates	The samples were analysed at day 0 (one sample), 1 (in duplicate), 3 (in duplicate), and 5 (in duplicate) as described in OECD guideline 111.
Sampling	Day 0, 1, 3 and 5.
Analytical parameter	LSC measurements were performed using a Packard Tri-Carb liquid scintillation analyzer in duplicate. A validated HPLC-MS/MS-method for the determination of the test item was used in order to determine the “lead components” of the complex test item. (HPLC equipment: Dionex Summit with Raytest Ramona ^{14}C-detector Column Luna C18(2), 100x 2,0 mm, 3µm 100 A° (Phenomenex). From the HPLC runs the fractions containing radioactive peaks were sampled and subjected to the identification procedure. The MS/MS-methodology is described in detail in reference A4.2/02.
Preliminary test	Yes Since less than 10 % of the test item hydrolysed at 50 °C at pH 4, 7, and 9 within 5 days, corresponding to a DT_{50} (25°C) > 1 year (tier 1), no further hydrolysis testing was considered to be necessary (see below).

Section A7.1.1.1.1
Annex Point IIA 7.6.2.1

Hydrolysis as a function of pH and identification of breakdown products

Results

Concentration and hydrolysis values	Recoveries of ^{14}C from the test solutions decreased to 59.3 % (for pH 7) and 58.6 % (for pH 9) at day 5 of the experiment respectively, whereas at pH 4, 105.6 % could be recovered. Low recoveries at pH 7 and 9 were explained by adsorption of the test substance onto the walls of the glass vials. Rinsing of the vial walls with 1 ml acetonitrile/water (3:7, v/v) and sonification resulted in improved recoveries of close to 100 % (see Table A7.1.1.1.1- 7). Significant chromatographic peaks other than those attributable to the lead components of the test item were not detected: Any other signals accounted for less than 2.5 % ITR at any sampling time.
Hydrolysis rate constante (k_h)	Not applicable because the preliminary test results indicate that the substance is hydrolytically stable.
Dissipation time	Not applicable because the preliminary test results indicate that the substance is hydrolytically stable.
Concentration-time data	A graph is not presented in view of the hydrolytic stability of the test substance.
Specification of the transformation product	Not applicable because the test results indicate that the substance is hydrolytically stable.

Applicant's Summary and conclusion

Materials and methods	Dodecyl-1-^{14}C-labelled Ampholyt 20/100 was tested for hydrolysis according to OECD guideline 111. The test item was dissolved in buffered aqueous media of defined pH-values (pH 4, 7, or 9) and maintained at constant temperature (50 °C) in the darkness. The concentrations of the lead compounds of Ampholyt 20/100 were measured as a function of time. At 0, 1, 3 and 5 days radioactivity as well as the identity of the components of Ampholyt 20/100 and potential hydrolysis products were verified by HPLC-MS/MS. The results of the preliminary test indicated no need for further testing. Deviations from the guidelines were not reported.
Results and discussion	From day 1 to day 5 of the experiment, recoveries of ^{14}C in the test solution decreased from approx. 100 % to 59.3 % (at pH 7) and 58.6 % (at pH 9), respectively, whereas at pH 4 105.6 % of the radioactivity were recovered on day 5. It was clearly demonstrated that the test material had adsorbed onto the walls of the glass vials at pH 7 and 9, but was removable by rinsing the vial walls with acetonitrile/water, leading to total recovery rates of 92.9 and 102% as the sum of the test item solutions and the rinsing solutions. Less than 10 % of the test item hydrolysed in the preliminary test during five days at 50 ± 5 °C. Hence, k_H and DT_{50} or DT_{90} values could not be established. In conclusion, Ampholyt 20/100 is considered to be hydrolytically stable. The half-life periods at pH 4, 7 and 9 can be expected to exceed one year at 25 °C.

Section A7.1.1.1.1 Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA 7.6.2.1

k_H	n.d.
DT_{50}	> 1 year (Tier 1)
r^2	n.d.
Conclusion	The substance Ampholyt 20/100 can be characterised as being hydrolytically stable.
Reliability	1
Deficiencies	None

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	17/12/12
Materials and Methods	Applicants version is considered acceptable with the following comments/additions: **Table CA 7.1.1.1.1- 1** gives the Structural formula, composition and labelling position of the test item Ampholyt 20/100 (^{14}C-labelled). The buffer solutions were sterilised by sterile filtration (Whatman, FP30/0.2; CA-S, 0.2 µm, 7 bar max) in order to exclude biodegradation. It is unclear if sterility was maintained throughout the course of the study. However, this is deemed acceptable in this case as the test substance was observed to be stable over the course of the incubation period. Exact pH values measured were 4.09, 7.05 and 9.16. The nominal concentration was 0.0294 g/L. The water solubility of Ampholyt 20 is reported as ≥200 g/L. The test solution concentrations did not exceed water solubility. The concentration of the organic solvent (ethanol) was 1% by volume in the test solution. For lead component 5, no reference substance was available. Instead, the substance was identified by the daughter ion at 359.5 m/z. The Reviewer notes the peak for [redacted] is not resolved.
Results and discussion	The applicants version is deemed acceptable with the following additions: The pattern of radioactivity of the test solution (components 1-5) expressed as relative peak area is approximately constant within 5 d of the preliminary hydrolysis study. Minor amount amounts of two unidentifiable residues were detected, but not exceeding 2.5 %ITR at any time. At pH 9 decreasing recovery of ^{14}C was primarily due to disappearance of component [redacted] **Table CA 7.1.1.1.1- 2**). At the same time there was a slight change in the relative peak area of the [redacted] was observed.
Conclusion	The applicants version is deemed acceptable
Reliability	1
Acceptability	Study is deemed acceptable
Remarks	

	COMMENTS FROM ...
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Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	

Remarks	

Table A7.1.1.1.1- 4: Type and composition of buffer solutions; to avoid oxidation processes dissolved oxygen was removed by bubbling with argon for 5 minutes.

Exact pH-values measured	Type of buffer (final molarity)	Composition
4.09	The buffer concentration did not exceed 0.01 M	Commercially available citrate buffer
7.05	The buffer concentration did not exceed 0.01 M	Buffer of potassium citrate monobasic solution adjusted by NaOH was applied instead of phosphate, since with phosphate buffer precipitation may be expected from previous experience with the substance
9.16	The buffer concentration did not exceed 0.01 M	Commercially available borate buffer

Table A7.1.1.1.1- 5: Description of test solution.

Criteria	Details
Purity of water	The buffer solutions were sterilized by sterile filtration (Whatman, FP 30/0.2; CA-S, 0.2 µm, 7 bar max) in order to exclude biodegradation
Preparation of test medium	The test substance (147.0 mg; 209.0 MBq according to the certificate) was dissolved in 50 mL ethanol, resulting in a calculated concentration of the stock solution of 2.94 g/L. 100 µL of the stock solution was added to 10 mL buffer solution at pH 4, 7, and 9, respectively
Test concentrations	Aliquots were analysed for radioactivity using LSC: pH = 4: 38.68 KBq/mL, equivalent to 27.18 µg test item/mL pH = 7: 37.64 KBq/mL, equivalent to 26.45 µg test item/mL pH = 9: 37.85 KBq/mL, equivalent to 26.60 µg test item/mL
Temperature [°C]	50°C ± 0.1 °C
Controls	None
Identity and concentration of co-solvent	None
Replicates	Duplicate sampling (at day 0 only one sample)

Table A7.1.1.1.1- 6: Description of test system.

Glassware	Sample vials for HPLC analysis
Other equipment	None
Method of sterilization	Sterile filtration of the buffer solutions (Whatman, FP 30/0.2; CA-S, 0.2 µm, 7 bar max)

Table A7.1.1.1.1- 7: Hydrolysis of test compound, transformation products and reference substance, expressed as percentage of initial concentrations, at pH 4, pH 7 and pH 9. n.d. = not determined.

Buffer	Sampling time [h]	Replicate	Concentration of ^{14}C-test item [µg/mL]	Recovery of applied radioactivity [%]	Recovery of applied radioactivity [%] in washing solution	^{14}C-test item concentration in washing solution [µg/mL]	Sum: µg ^{14}C-test item/mL	Sum: recovery of applied radioactivity [%]
pH 4								
	0	0d	27.18	**100.00**				
	24	1d/1	27.65	**101.75**				
		1d/2	27.98	**102.94**				
	72	3d/1	27.63	**101.68**				
		3d/2	28.31	**104.16**				
	120	5d/1	28.24	**103.91**				
		5d/2	29.14	**107.20**				
pH 7								
	0h	0d	26.45	**100.00**				
	24h	1d/1	18.80	**71.06**	29.51	7.81	26.6	**100.6**
		1d/2	19.16	**72.44**	29.53	7.81	27.0	**102.0**
	72h	3d/1	16.20	**61.23**	34.14	9.03	25.2	**95.4**
		3d/2	17.39	**65.74**	34.43	9.11	26.5	**100.2**
	120h	5d/1	15.61	**59.01**	38.09	10.07	25.7	**97.1**
		5d/2	15.77	**59.62**	38.19	10.10	25.9	**97.8**
pH 9								
	0h	0d	26.60	**100.00**				
	24h	1d/1	20.37	**76.58**	20.70	5.51	25.9	**97.3**
		1d/2	18.30	**68.79**	28.36	7.54	25.8	**97.1**
	72h	3d/1	18.25	**68.60**	26.56	7.06	25.3	**95.2**
		3d/2	16.22	**60.97**	34.32	9.13	25.3	**95.3**
	120h	5d/1	16.34	**61.42**	31.89	8.48	24.8	**93.3**
		5d/2	14.84	**55.79**	37.12	9.87	24.7	**92.9**

Applied radioactivity:

pH = 4: 38.68 KBq/mL, equivalent to 27.18 µg test item/mL

pH = 7: 37.64 KBq/mL, equivalent to 26.45 µg test item/mL

pH = 9: 37.85 KBq/mL, equivalent to 26.60 µg test item/mL.

Evaluation by the Competent Authority

Table CA 7.1.1.1.1-1: Structural formula, composition and labelling position of the test item Ampholyt 20/100 (^{14}C-labelled)

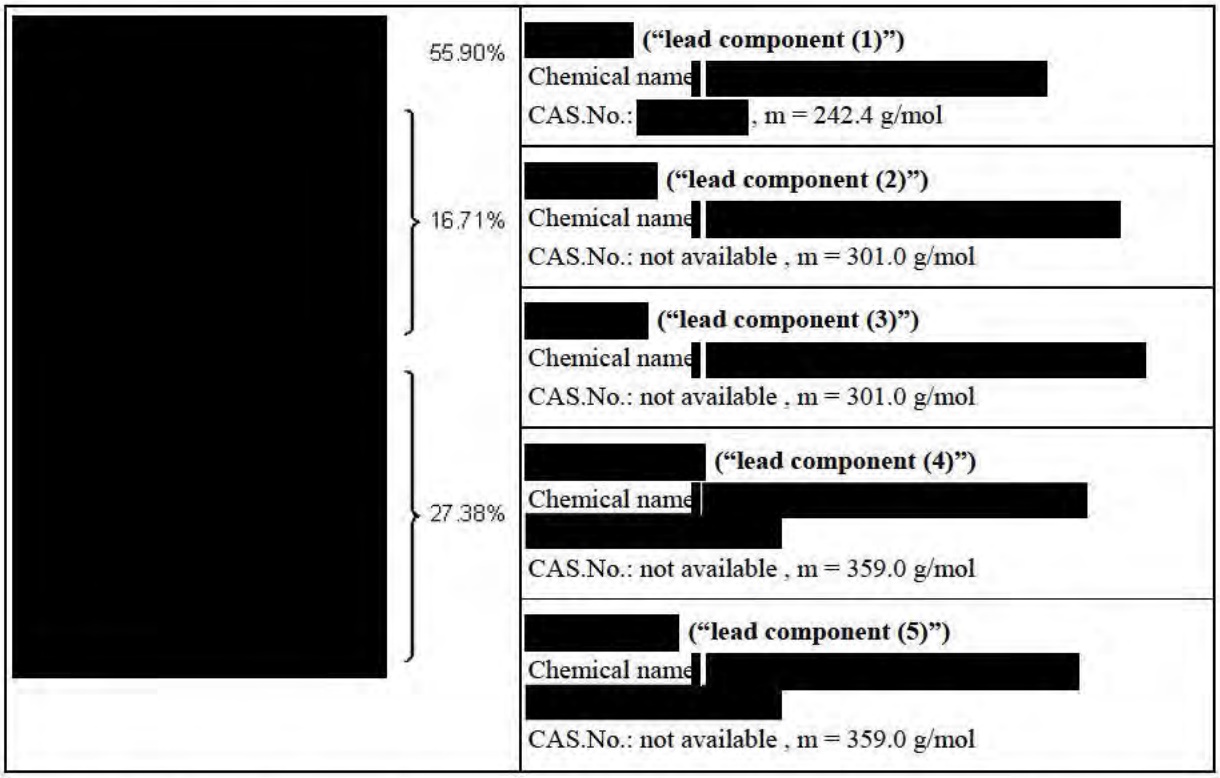


Table CA 7.1.1.1.1- 2. Summary of recovery of single substances (mean values of two duplicates per sampling, in kBQ/mL

	sampling	0d	1d	3d	5d
pH 4		23,3	23,0	23,2	23,3
		12,3	12,1	12,7	12,3
		0,9	0,9	1,1	1,1
		2,1	3,0	2,9	3,3
	unknown		0,4		0,4
	unknown		0,2		0,3
pH 7		21,5	21,3	20,3	20,4
		13,0	11,9	12,1	11,9
		1,0	1,0	0,8	1,0
		2,2	3,2	3,0	2,7
	unknown		0,4	0,4	0,3
	unknown		0,3	0,3	0,2
pH 9		22,7	20,5	18,7	17,7
		12,1	11,0	11,0	10,7
		1,0	1,0	1,0	0,9
		1,9	2,8	3,2	3,4
	unknown		0,7	1,1	1,3
	unknown		0,7	1,1	1,3

Section A7.1.1.1.2 **Phototransformation in water including identity of transformation products**
Annex Point IIA 7.6.2.2

Official use only

1. Reference

1.1	**Reference**	**A7.1.1.1.2/01**: [redacted] Direct phototransformation of Ampholyt 20/100 in water according to the draft OECD-guideline "Phototransformation of Chemicals in Water – Direct and Indirect Photolysis", and SETAC procedures. Fraunhofer-Institute for Molecular Biology and Applied Ecology, Schmallenberg Germany, report No. EBR-013/7-05, November 17, 2008 (unpublished).
1.2	**Data protection**	Yes
1.2.1	Data owner	Goldschmidt GmbH
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. for the purpose of its entry into Annex I.

2. Guidelines and Quality Assurance

2.1.	**Guideline study**	Yes Draft OECD Guideline "Phototransformation of Chemicals in Water – Direct and Indirect Photolysis" (August 2000) SETAC procedures
2.2.	**GLP**	Yes
2.3.	**Deviations**	As the test item comprises 5 different lead components the UV/VIS-absorption in the range of 290-800 nm depending on pH was not recorded, since it would not provide any information on the phototransformation of the single substances.

3. Materials and Methods

3.1	**Test material**	^{14}C-labelled Ampholyt 20/100 (Dodecyl-1-^{14}C-labelled)
3.1.1.	Lot/Batch number	XVI/38
3.1.2	Specification	Ampholyt 20 is a reaction product (obtained as a "product by process" in form of a 20 % aqueous solution) of alkyl-oligoaamines with chloroacetic acid, the spectrum of alkyl chain lengths ranging from C_{10} to C_{16}. The dodecyl group (C_{12}) dominates the chain length spectrum by approx. 75 %. Ampholyt 20/100 is obtained from Ampholyt 20 by lyophilisation exclusively for testing purposes, thus simply constituting anhydrous Ampholyt 20. The radiolabelled material used for this study, dodecyl-1-^{14}C-Ampholyt 20/100, was deliberately restricted to the C_{12} moiety only, which is considered as representative for the complex active substance. Satisfactory compliance of the labelled substance's composition with that of technical active substance was confirmed by TLC.

Section A7.1.1.1.2 Phototransformation in water including identity of transformation products

Annex Point IIA 7.6.2.2

3.1.3	Purity	Not defined (mixture of amines, dodecyltrimethylenedi-, reaction products with chloro-acetic acid); however, the signals attributable to the dodecyl-components of Ampholyt 20 account for 94.86% of the radioactivity of the test item. The test material may therefore be regarded as sufficiently pure.
3.1.4	Radio-labelling	Dodecyl-1-^{14}C-labelled. The structural formula, composition and labelling position are given in Table A7.1.1.1.2- 1. Specific activity: 1423 KBq/mg
3.1.5	UV/VIS absorption spectra and absorbance value	The absorbance of the chemical actinometer solution was recorded by a Varian Cary 1 spectrophotometer. As the test item comprises 5 different lead components the UV/VIS-absorption in the range of 290-800 nm depending on pH was not recorded, since it would not provide any information on the single substances.
3.1.6	Further relevant properties	The a.s. is a multi-component substance as specified in Section A2.
3.2	**Reference substance**	Not stated
3.3	**Test solution**	Please refer to Table A7.1.1.1.2- 2.
3.4.	**Testing procedure**	
3.4.1.	Test system	Details are given in Table A7.1.1.1.2- 3.
3.4.2.	Properties of light source	Please refer to Table A7.1.1.1.2- 3.
3.4.3	Determination of irradiance	Please refer to Table A7.1.1.1.2- 3 and Figure A.7.1.1.1.2- 1.
3.4.4.	Temperature	Please refer to Table A7.1.1.1.2- 2.
3.4.5	pH	5, 7, and 9
3.4.6	Duration of the test	7 days of irradiation
3.4.7.	Number of replicates	3
3.4.8.	Sampling	Solutions were sampled at 0, 1, 2, 3, 5 and 7 days of continuous irradiation and analysed.

3.4.9	Analytical methods	At days 0, 1, 2, 3, 5, and 7 the mass balance (^{14}C) as well as the identity of the five "lead components" of Ampholyt 20/100 and potential photolysis products were assayed using LSC, radio-HPLC and LC-MS/MS. The mass balance was obtained by analysis of the radioactivity prior to and after irradiation. An aliquot of 50 µL was used for LSC-analysis. Another 1 mL was transferred into a HPLC-vial and analysed by radio-HPLC without any further clean-up. For determination of test item adsorbed onto the glass walls of the test vessels, the vessels were rinsed with 2 mL of acetonitrile/water mixture (3:7 v:v) containing 0.5% formic acid. The test vessel with the solvent was treated in an ultrasonic bath for 5 minutes. The rinsing solution was then analysed by LSC and radio-HPLC without any further work-up.
3.5	**Transformation products**	
3.5.1	Method of analysis for transformation products	To trap volatile transformation products the vials were flushed by a gentle stream of CO_2-free synthetic air. The outgoing gas was bubbled through three absorption traps in sequence containing ethylenglycol, 0.5 N H_2SO_4 and 1 N NaOH in order to trap volatile metabolites and to determine the rate of mineralisation (quantitation of $^{14}CO_2$), (screening test over a period of 48 hours). Transformation products were detected by LSC, radio-detection (radio-HPLC) and LC-MS/MS.

4.Results

4.1	**Screening test**	In the initial screening test it was demonstrated by mass balance of the applied radioactivity that during irradiation for 48 hours no significant volatilisation of test substance occurred.
4.2	**Actinometer data**	A p-nitroanisole/pyridine actinometer was used, exposed to light at wavelengths above 290 nm to calculate the half life for the actinometer. The irradiated solutions were analysed for p-nitroanisole and quantified by HPLC/UV. The start concentration ratio of p-nitroanisole/pyridine was at least 1:10 to maintain an excess of pyridine throughout the reaction (nominal start-concentrations: p-nitroanisol 10^{-5} mol/L, pyridine 10^{-4} mol/L and 10^{-3} mol/L). According to Dulin and Mill the quantum yield of the reaction is independent of the irradiation wavelength. It solely depends on the concentration of pyridine, following the relationship: $\phi_{Act} = 0.44\ [pyr] + 0.00028$ Based on this equation the quantum yield of the actinometer used should have been 3.24×10^{-4} and 7.2×10^{-4}, respectively. The results are summarised in Table A7.1.1.1.2- 4.
4.3	**Controls**	Dark controls
4.4	**Photolysis data**	
4.4.1	Concentration values	Not applicable; in view of the nature of the active substance, constituting a multi-component substance, molar concentrations could not be determined.

Section A7.1.1.1.2 Phototransformation in water including identity of transformation products

Annex Point IIA 7.6.2.2

4.4.2	Mass balance	The distribution of dissolved and adsorbed radioactivity clearly depended on the pH of the solution. Solubility of the test item in water is known to be increased at acidic pH. At pH 5 the amount of radioactivity adsorbed onto the glass walls was constantly in the range of 5 to 7 % ITR. In contrast, at pH 9 the adsorbed radioactivity ranged from about 20 to 40 % ITR. The material was removable by rinsing the dish walls with acetonitrile/water. The ^{14}C-recovery was always in the range of 90 % to 110 % ITR with the exception of three single samples (88.4 %, 113.2 % and 129 %). These samples were all found at pH 9 experiments, where substance solubility has been shown to be limited, which may have caused determination errors. However, these deviations do not affect the overall result of the study. Thus, the mass balance is considered to be complete.	
4.4.3	k^c_p	Not applicable	
4.4.4	Kinetic order	Not determined	
4.4.5.	k^c_p / k^a_p	Not applicable	
4.4.6.	Reaction quantum yield ($\phi^c E$)	Not applicable	
4.4.7.	$k_p E$	Not applicable	
4.4.8.	Half-life ($t_{1/2E}$)	Neither a theoretical photolysis half life nor the quantum yield could be determined since it is not possible to determine substance specific molar absorption coefficients for the mixture. However, over a period of five days of irradiation the pattern of the five lead components expressed as relative peak area of the radio-HPLC did not change significantly in aqueous solutions when compared to the respective dark controls. Minor amounts of unidentified signals were detected in both, the irradiated samples and the dark controls. Thus, a value for environmental half-life cannot be given; Ampholyt 20 is considered to be photolytically stable.	X1

Section A7.1.1.1.2 Phototransformation in water including identity of transformation products

Annex Point IIA 7.6.2.2

Specification of the transformation product

At the 7 day sampling two additional peaks were detected by radio-detection with an amount of 9.3 and 4.9 % ITR as a maximum. Both substances were detected in the irradiated samples at each pH tested. The transformation rate seems to be highest at pH 5. Both substances did not exceed the trigger for identification work of 10 % in any sampling or of 5 % in two consecutive samplings, respectively. At the same time the concentration of [redacted] decreased to almost 50 % of its starting concentration in the irradiated samples, initially suggesting that the substances detected may be transformation products of [redacted]. In addition an increase of the double substituted substances (di Gly) was found at the 7 day sampling.

Masses of 345 g/mol and 285 g/mol for the transformation products were determined by LC-MS/MS. However, this is inconsistent with regard to the observed [redacted] decrease, since the mass of [redacted] is only 242.4 g/mol. Thus, the identity of the transformation products could not be clarified. However, since their concentrations were always below the threshold for "major transformation products", further identification work is not required.

Both transformation products were observed in the rinsing solutions and in the dark controls, suggesting that they may originate from processes other than phototransformation, e.g. surface catalysis on the vessel walls.

Applicant's Summary and conclusion

Materials and methods

The test item Ampholyt 20/100, represented by five ^{14}C-labelled "lead components" sharing the dodecyl moiety, was subjected to aqueous photolysis at pH 5, 7 and 9. Samples were irradiated for 7 days in the SUNTEST device at continuous irradiation, corresponding to 30 days of natural summer sunlight (day/night rhythm) as determined by chemical actinometry. At days 0, 1, 2, 3, 5, and 7 the radioactivity balance as well as the identity of five "lead components" of Ampholyt 20 and of potential photolysis products were assayed using LSC, radio-HPLC and LC-MS/MS.

Section A7.1.1.1.2 Phototransformation in water including identity of transformation products

Annex Point IIA 7.6.2.2

Results and discussion	Up to day 7 recoveries of ^{14}C were in the range of 90 to 110% with the exception of three samples. These samples were all found at pH 9 experiments, where substance solubility has been shown to be limited. It was figured that the test material adsorbed onto the walls of the glass dishes used for the irradiation experiment. The material was removable by rinsing the dish walls with acetonitrile/water. From the HPLC analysis of the test solutions it is concluded that the pattern of the five lead components expressed as relative peak area is approximately constant until the 5 day sampling in the irradiated samples as well as in the respective dark control. This was confirmed by positive identification of the signals by LC-MS/MS. Minor signals appeared in the radio-HPLC chromatogram which could not be attributed to the test item but did not show a positive correlation to the irradiation interval applied, either. The concentrations of the transformation products determined in the test solutions did not follow a common trend. Except the fact that the concentrations at the 7d samplings are generally highest, their concentrations at other samplings vary. In addition, both transformation products were also detected in the rinsing solutions and in the dark controls. At each pH tested one of the three replicates showed only very low or even no transformation at all at the 7 d sampling. These findings indicate that processes other than phototransformation may be responsible for the occurrence of these substances, e.g. surface catalysis on the vessel walls. Based on the data obtained no kinetics for direct phototransformation could be determined.
k^c_p	Not applicable
k_pE	Not applicable
ϕ^cE	Not applicable
$t_{1/2E}$	Not applicable
Conclusion	It is concluded that direct phototransformation over a period of 30 days of natural summer sunlight is an insignificant process for Ampholyt 20/100 within the environmentally relevant pH range of natural waters between pH 5 and 9. On the basis of these results, direct photolysis in water cannot be considered to contribute significantly to abiotic degradation in aqueous systems under environmentally relevant conditions. This study is considered to be valid without restrictions.
Reliability	1
Deficiencies	None

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	18/12/12	
Materials and Methods	Applicants version is considered acceptable with the following comments: All glassware and reaction vessels/photolysis cells used in the study were sterilised by autoclaving before use. It is unclear from the report if sterility was maintained during the course of the incubation period. The concentration of the organic solvent (ethanol) was 1% by volume in the test solution. It is not reported if oxygen was excluded from the buffer solutions. Temperature was controlled in the experiment at 20°C.	

Results and discussion	Applicants version is considered acceptable with the following comments: The mass balance is considered acceptable, (**Table CA7.1.1.1.2- 1).** The CA notes in one sample (pH 9, 3 d/1) ~80%of the radioactivity was found in the rinsing solution. 50.85 %AR was found in solution. This sample was considered as an outlier and was not considered for further evaluations. X1. The CA notes slightly higher radioactivity associated with unknowns was observed in the irradiated samples Over a period of 5 d of irradiation the pattern of the components did not change significantly in aqueous solution when compared to the respective dark controls , Table CA 7.1.1.1.2- 2 to Table CA 7.1.1.1.2- 4. Minor amounts of unidentified signals were detected in both, the irradiated samples and the dark controls. Transformation products detected exclusively in the rinsing solutions may be due to surface catalysed reactions on the vessel walls, since no correlation to the irradiation period could be observed. These substances were detected in the dark controls. However the amounts were lower except in the case of pH 7 where similar percentages were observed in the light and dark samples. The identity of the transformation products could not be established. Unknown 1 and 2 reached up to 9.3% (**Table CA 7.1.1.1.2- 2** and 4.9 % (**Table CA 7.1.1.1.2-3)** of the initial applied radioactivity (IR) in day 7 samples. The highest levels were observed at pH 5. The lowest levels were observed at pH 7. Both substances did not exceed the EU trigger value of 10 %AR. The CA notes over this time period there was significant decrease in substance 1 and 2+3 (Table CA 7.1.1.1.2- 2) and a significant increase in substance 4 and 5. Minor changes were observed in the dark control.

	Over a period of 5 d of irradiation the pattern of the components of the test solution did not change significantly in aqueous solution when compared to the respective dark controls. However, in the day 7 samples the composition of the test mixture appeared to change relative to dark controls. Components 1 and 2+3 decreased. Components 4 and 5 increased. Only minor changes were observed in the dark control. No significant transformation product greater than 10 % was observed in solution**.** The study author states the replicates of the 7 d samplings are not reproducible. According to the study author *'at each pH tested one of the three replicates showed only very low or even no transformation at all at the 7 d sampling. These findings indicate that processes other than photo transformation may be responsible for the occurrence of these substances e.g. surface catalysis on the vessel walls.'* **Figure CA. 7.1.1.1.2-1** to **Figure CA. 7.1.1.1.2**-4 shows the composition the reproducibility of the chromatograms at pH 4, 7 and 9 on day 7 In the initial screening test it was demonstrated that during irradiation for 48 hr no significant volatilisation of the test substance (maximum observed radioactivity was 0.13 %AR in NaOH traps, **Table CA 7.1.1.1.2- 5**)
Conclusion	Over a period of 5 d of irradiation the pattern of the components of the test solution did not change significantly in aqueous solution when compared to the respective dark controls. However, in the day 7 samples the composition of the test mixture appeared to change relative to dark controls. Components 1 and 2+3 decreased. Components 4 and 5 increased. Only minor changes were observed in the dark control. No significant transformation product greater than 10 % was observed in solution. If direct photolysis were occurring the composition of the reaction mixture during the first five days would be expected to change. However, as pointed out by the study author the reaction mixture only changed during the last two days. The replicates from this period (7 d) are not reproducible. Consequently, the study authors suggestion that other processes apart from direct photolysis may be at play seems plausible
Reliability	2
Acceptability	Study is deemed acceptable.
Remarks	

	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.1.1.1.2- 1: Structural formula, composition and labelling position of the test item Ampholyt 20/100 (^{14}C-labelled).

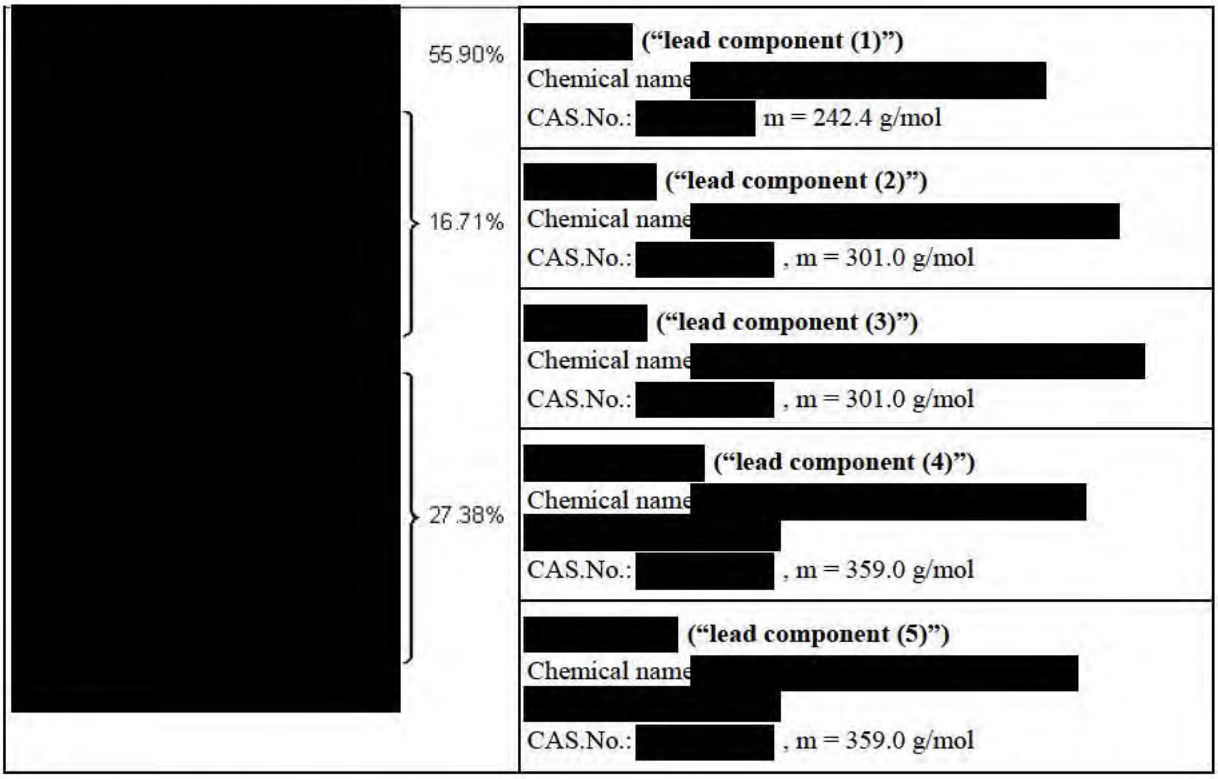


Table A7.1.1.1.2- 2: Description of test solution and controls.

Criteria	Details
Purity of water	Not stated
Preparation of test chemical solution	^{14}C-radioactively labelled Ampholyt 20/100 was dissolved in buffered aqueous media of defined pH-values (pH 5, 7 or 9).
Test concentrations [mg a.s./L]	pH 5: 31.27 mg/L (445.00 KBq/10 mL test solution) pH 7: 29.12 mg/L (414.32 KBq/10 mL test solution) pH 9: 30.95 mg/L (440.36 KBq/10 mL test solution) The test concentrations were calculated based on the reported specific radioactivity of 1423 KBq/mg.
Temperature [°C]	20 °C during irradiation
Preparation of a.s. solution	For accurate dosing the test item first was dissolved in ethanol. The stock solution was pipetted into 10 mL buffer solutions (MERCK ready to use buffers) each of pH 5, 7, and 9. The concentration of the organic solvent was 1 % by volume in the test solution
Controls	Non-irradiated dark controls were kept under identical conditions to distinguish between photochemical and other transformation processes
Identity and concentration of co-solvent	Ethanol, 1 % by volume in the test solution.

Table A7.1.1.1.2- 3: Description of test system.

Criteria	Details
Laboratory equipment	UV/VIS: The absorbance of the chemical actinometer solution was recorded by a Varian Cary 1 spectrophotometer. LSC measurements were performed using a Packard Tri-Carb liquid scintillation analyzer. HPLC equipment: Dionex Summit with Raytest Ramona ^{14}C-detector, column: Luna C18(2), 100x 2.0 mm, 3 µm 100 A° (Phenomenex)
Test apparatus	
Properties of artificial light source:	Heraeus Suntest apparatus
Nature of light source	Polychromatic irradiation, light was produced by means of a Xenon arc lamp
Emission wavelength spectrum	The spectral distribution is given in Figure A.7.1.1.1.2- 1
Light intensity	Comparable to that of daylight (measured by an appropriate spectral radiometric sensor, non-GLP)
Filters	The light source was equipped with appropriate cut-off filters which ensure that the minimum wavelength of the irradiation will be at λ = 290 nm and the maximum wavelength at λ = 800 nm

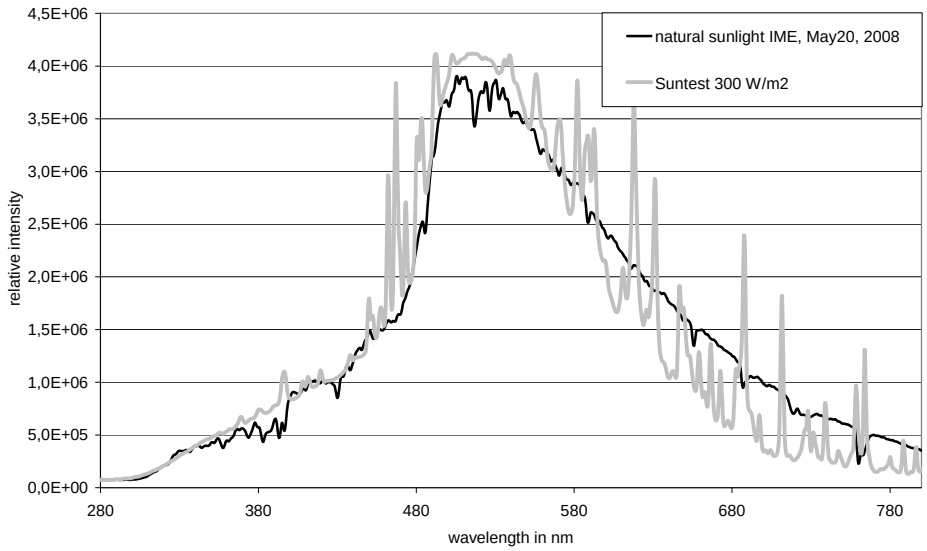


Figure A.7.1.1.1.2- 1: Spectral distribution of SUNTEST compared to natural sunlight

Table A7.1.1.1.2- 4: Screening test results; conversion into days of natural summer sunlight; results of chemical actinometry and ABIWAS calculation; half lives of chemical actinometer and transfer rate.

Pyridine concentration [mol/L]	Half life in days			Ratio		30 day equivalent	
	Suntest	40 °	55 °	40 °	55 °	40 °	55 °
10^{-3}	0.1262	0.564	0.593	4.5	4.7	6.7 d	6.4 d
10^{-4}	0.2945	1.25	1.32	4.2	4.5	7.1 d	6.7 d

Evaluation by the Competent Authority

Table CA 7.1.1.1.2- 1

Table 5: Recovery of applied radioactivity for irradiated sub-samples

Sampling time [d]	Test item sample	Recovery of applied radioactivity [%]					
		pH 5		pH 7		pH9	
		solution	rinsing	solution	rinsing	solution	rinsing
0	0d	100		100		100	
1	1d/1	95.52	5.22	90.35	8.52	87.03	18.86
	1d/2	94.54	5.91	90.83	10.15	82.46	24.00
	1d/3	96.41	4.93	93.00	8.64	87.13	19.17
	dark control	95.95	6.35	94.16	10.86	90.46	18.71
2	2d/1	96.70	4.65	91.35	7.80	82.95	21.05
	2d/2	96.13	4.72	91.48	9.76	85.52	21.66
	2d/3	96.96	4.96	95.45	6.97	88.43	19.40
	dark control	93.77	6.83	91.97	13.29	89.01	19.63
3	3d/1	94.58	4.98	93.94	6.99	*50.85**	*78.18**
	3d/2	96.38	4.72	94.96	6.65	77.45	29.94
	3d/3	92.10	6.83	95.35	7.21	77.94	35.24
	dark control	96.33	6.25	92.36	13.71	88.71	20.71
5	5d/1	90.25	6.82	91.47	8.40	69.95	18.41
	5d/2	91.34	6.12	91.03	8.80	76.04	25.42
	5d/3	93.81	5.84	93.62	7.23	74.51	24.71
	dark control	93.26	6.26	91.20	16.35	80.60	20.31
7	7d/1	93.62	5.48	91.50	7.87	77.25	24.90
	7d/2	92.53	6.35	78.19	14.95	51.56	41.31
	7d/3	92.94	6.41	77.72	16.11	60.21	41.14
	dark control	94.80	8.41	90.95	16.10	79.07	21.07

* *outlier, not considered for further evaluation*

Table CA 7.1.1.1.2- 2.

Table 6: Amount of components [% ITR] at pH 5 after irradiation; figures in italics denominate signals that were detected in rinsing solutions from vessel walls only.

Component	Distribution of radioactivity as determined by radio-HPLC at pH 5					
	start	1d	2d	3d	5d	7d
[redacted]	58.90*	57.72	58.23	55.98	48.96	30.29*
	31.73*	31.68	31.04	30.90	30.27	22.49*
	2.58*	2.50	2.63	3.31	3.79	6.74*
	6.78*	6.55	8.17	7.99	8.97	20.46*
u.i.1	--	*1.24*	*0.60*	0.45	4.15	9.32
u.i.2	--	*0.44*	*0.32*	1.97	*0.54*	2.56
u.i.3	--	*0.47*	*0.11*	*0.17*	*0.11*	3.13
u.i.4	--	*0.13*	*0.15*	*0.09*	*0.10*	1.07
u.i.5	--	*0.17*	--	*0.09*	*0.23*	0.77
u.i.6	--	*0.05*	--	*0.05*	--	0.74
u.i.7	--	*0.10*	--	--	--	--

*Identity confirmed by LC-MS/MS analysis of respective radio-HPLC eluent
u.i. = unidentified signal at radio-HPLC
-- below limit of determination (0.05 % ITR)

Table 7: Amount of components [% ITR] at pH 5, dark controls; figures in italics denominate signals that were detected in rinsing solutions from vessel walls only.

Component	Distribution of radioactivity as determined by radio-HPLC at pH 5, dark controls				
	1d	2d	3d	5d	7d
[redacted]	61.17	58.58	60.24	59.10	58.55*
	30.71	30.95	32.00	29.35	31.41*
	2.85	2.79	2.69	2.68	3.78*
	6.42	6.62	7.18	6.94	8.48*
u.i.1	*0.94*	*1.47*	*0.47*	*0.48*	*1.00*
u.i.2	--	--	--	*0.48*	--
u.i.3	--	--	--	*0.50*	--
u.i.4	--	--	--	--	--
u.i.5	--	--	--	--	--
u.i.6	--	--	--	--	--
u.i.7	--	--	--	--	--

*Identity confirmed by LC-MS/MS analysis of respective radio-HPLC eluent
u.i. = unidentified signal at radio-HPLC
-- below limit of determination (0.05 % ITR)

Table CA 7.1.1.1.2- 3.

Table 8: Amount of components [% ITR] at pH 7 after irradiation; figures in italics denominate signals that were detected in rinsing solutions from vessel walls only.

Component	Distribution of radioactivity as determined by radio-HPLC at pH 7					
	start	1d	2d	3d	5d	7d[1]
[redacted]	58.84*	57.90	56.44	59.13	56.79*	34.88*
	33.72*	31.37	29.83	31.04	30.19*	27.43*
	2.03*	2.81	2.05	2.28	2.16*	1.73*
	5.41*	6.18	6.33	6.14	6.21*	14.73*
u.i.1	--	*0.24*	2.50	1.36	2.76	5.53
u.i.2	--	*0.21*	2.15	1.18	*2.06*	4.86
u.i.3	--	*0.23*	*0.45*	*0.46*	--	*1.86*
u.i.4	--	*0.31*	*0.38*	*0.29*	--	--
u.i.5	--	*0.49*	*0.59*	*0.09*	--	--
u.i.6	--	*0.52*	*0.21*	--	--	--
u.i.7	--	*0.20*	*0.07*	--	--	--

*Identity confirmed by LC-MS/MS analysis of respective radio-HPLC eluent
[1] significant variation in replicates, see annex 3c
u.i. = unidentified signal at radio-HPLC
-- below limit of determination (0.05 % ITR)

Table 9: Amount of components [% ITR] at pH 7, dark controls; figures in italics denominate signals that were detected in rinsing solutions from vessel walls only.

Component	Distribution of radioactivity as determined by radio-HPLC at pH 7, dark controls				
	1d	2d	3d	5d	7d
[redacted]	62.20	62.12	62.56	62.43	62.91*
	32.94	29.01	30.83	31.78	32.02*
	2.06	3.75	2.73	2.85	3.15*
	5.84	6.28	7.74	8.25	6.79*
u.i.1	*0.41*	2.72	1.92	1.91	1.28
u.i.2	*0.52*	1.39	*0.29*	0.34	0.89
u.i.3	*0.13*	--	--	--	--
u.i.4	*0.19*	--	--	--	--
u.i.5	*0.15*	--	--	--	--
u.i.6	*0.23*	--	--	--	--
u.i.7	*0.36*	--	--	--	--

*Identity confirmed by LC-MS/MS analysis of respective radio-HPLC eluent
u.i. = unidentified signal at radio-HPLC
-- below limit of determination (0.05 % ITR)

Table CA 7.1.1.1.2- 4.

Table 10: Amount of components [% ITR] at pH 9 after irradiation; figures in italics denominate signals that were detected in rinsing solutions from vessel walls only.

Component	Distribution of radioactivity as determined by radio-HPLC at pH 9					
	start	1d	2d	3d	5d	7d[1]
[redacted]	59.97*	58.29	58.87	60.59*	52.97	34.67*
	31.46*	29.53	28.69	26.99*	24.08	18.90*
	2.23*	2.84	2.70	2.71*	2.12	6.55*
	6.34*	10.14	9.78	15.29*	11.12	19.56*
u.i.1	--	3.00	2.65	4.83	2.43	7.80
u.i.2	--	1.98	1.80	5.76	3.63	4.08
u.i.3	--	*0.53*	*1.10*	*1.00*	--	*2.94*
u.i.4	--	*0.09*	*0.27*	*0.24*	--	*2.03*
u.i.5	--	--	--	--	--	--
u.i.6	--	--	--	--	--	--
u.i.7	--	--	--	--	--	--

*Identity confirmed by LC-MS/MS analysis of respective radio-HPLC eluent
[1] significant variation in replicates, see annex 3c
u.i. = unidentified signal at radio-HPLC
-- below limit of determination (0.05 % ITR)

Table 11: Amount of components [% ITR] at pH 9, dark controls; figures in italics denominate signals that were detected in rinsing solutions from vessel walls only.

Component	Distribution of radioactivity as determined by radio-HPLC at pH 9, dark controls				
	1d	2d	3d	5d	7d
[redacted]	65.84	60.50	63.67	59.59	55.74
	32.30	31.95	32.52	30.43	31.22
	2.77	3.27	4.95	2.99	2.44
	8.26	7.96	8.28	7.35	7.41
u.i.1	--	1.44	--	*0.55*	*0.38*
u.i.2	--	1.73	--	--	*0.23*
u.i.3	--	*1.41*	--	--	*0.41*
u.i.4	--	*0.37*	--	--	*0.97*
u.i.5	--	--	--	--	*1.34*
u.i.6	--	--	--	--	--
u.i.7	--	--	--	--	--

*Identity confirmed by LC-MS/MS analysis of respective radio-HPLC eluent
u.i. = unidentified signal at radio-HPLC
-- below limit of determination (0.05 % ITR)

Figure CA. 7.1.1.1.2-1

Test item composition at test start:

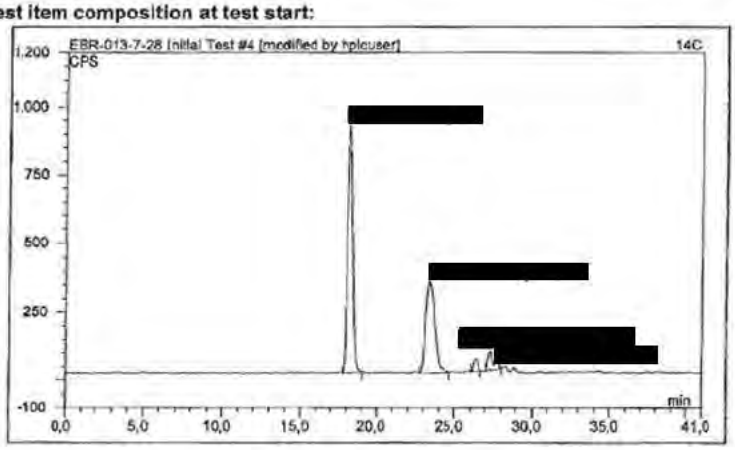


Figure 2: radio-HPLC chromatogram of the test item in aqueous solution

Radio-HPLC of rinsing solution

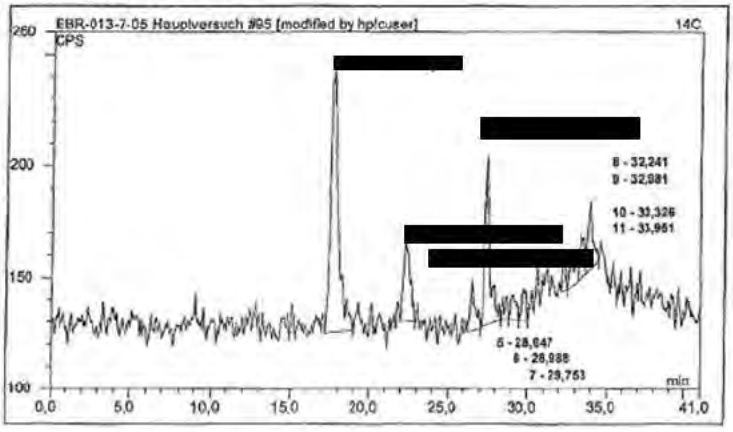
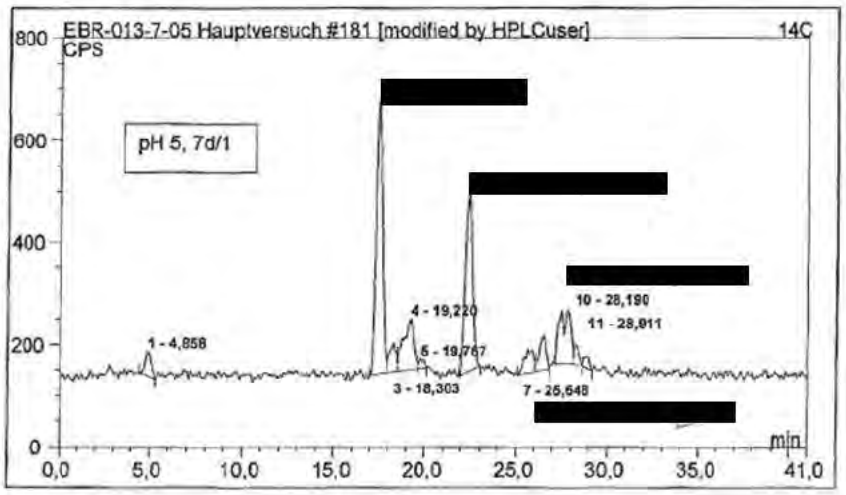
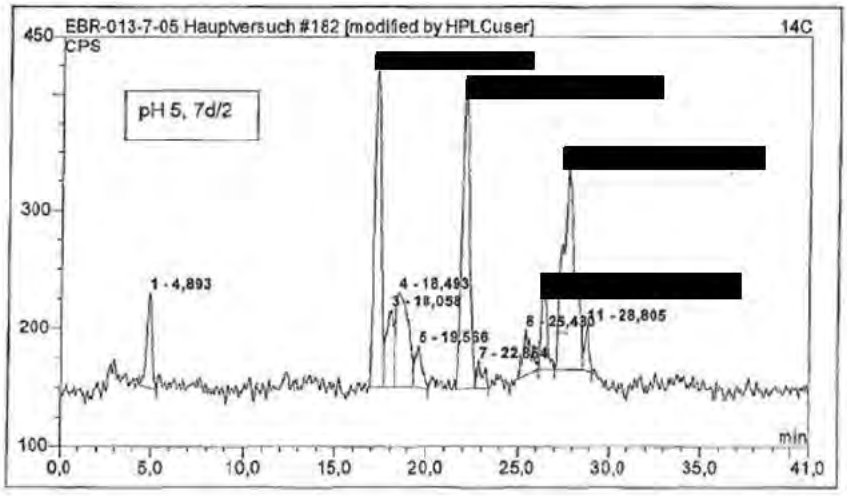


Figure CA. 7.1.1.1.2-2

Figure 3: example of radio-HPLC chromatogram of rinsing solution (1d/1, pH=7)
radio-HPLC at 7d sampling





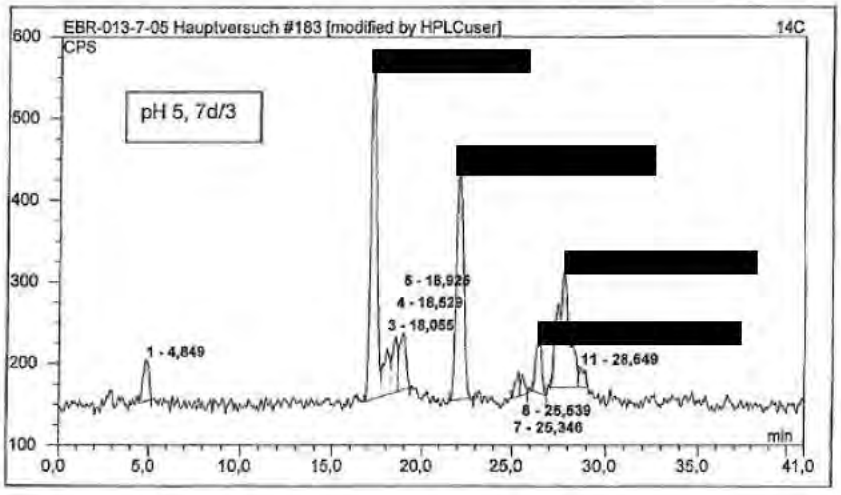


Figure CA. 7.1.1.1.2-3

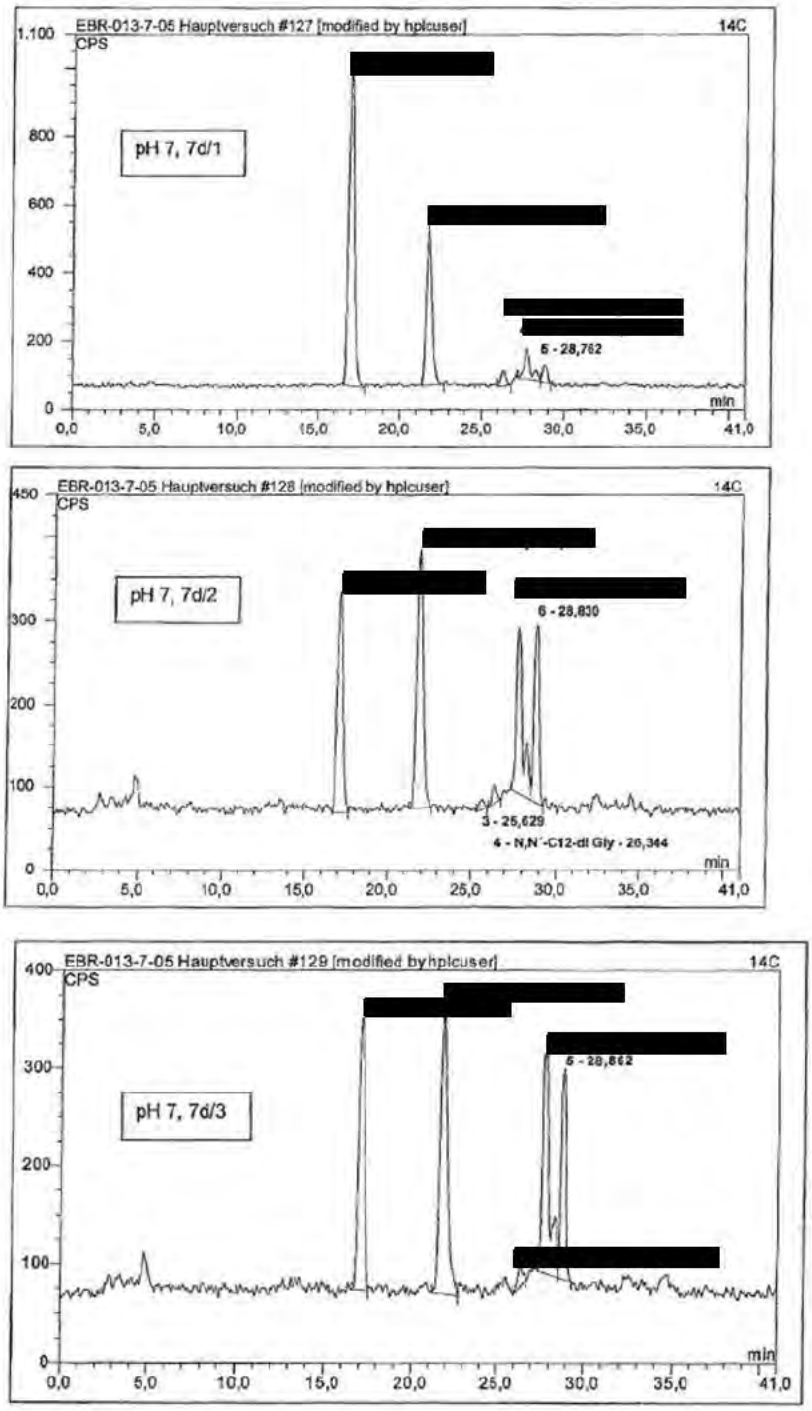
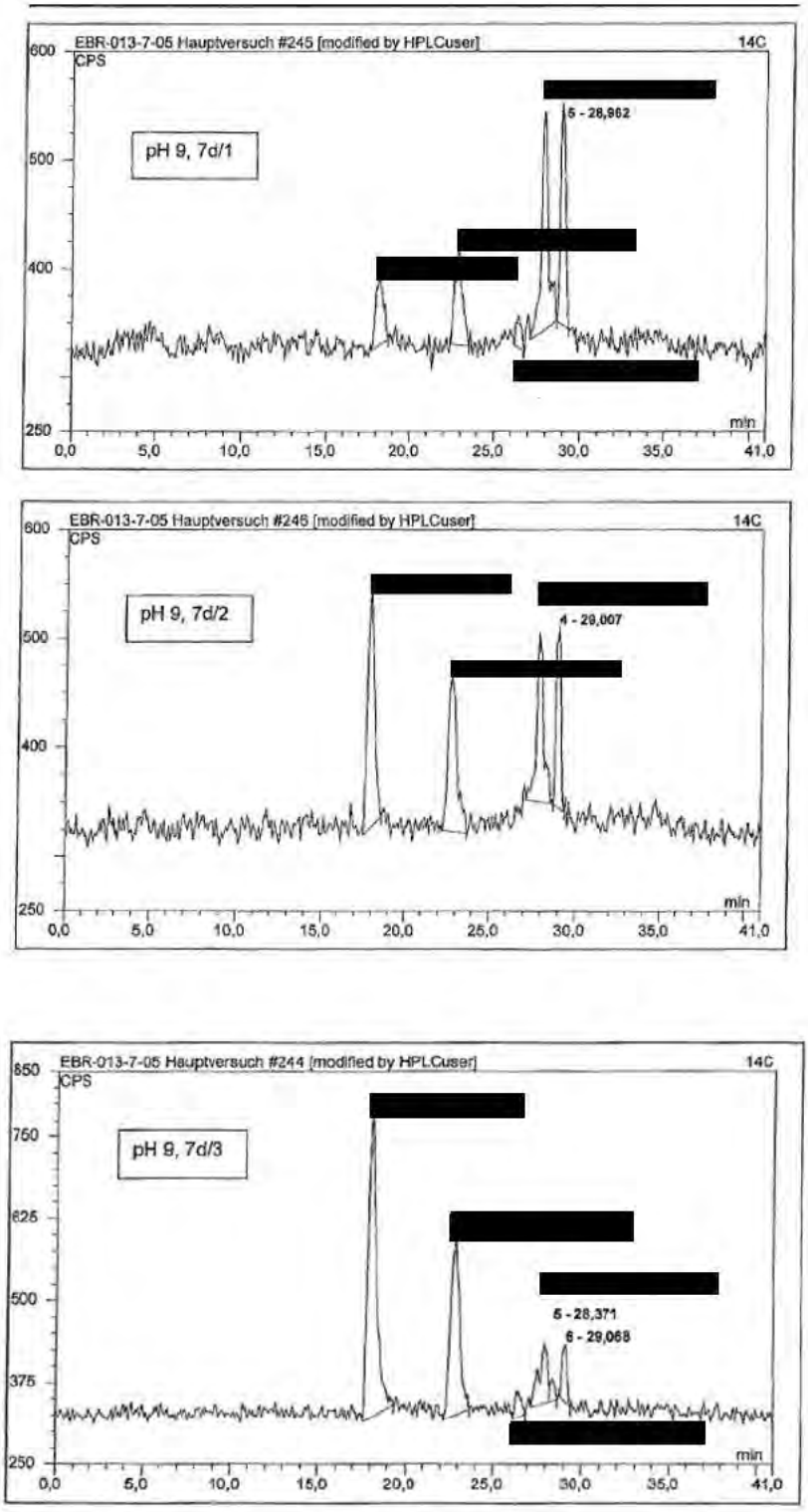


Figure 5: radio-HPLC of 7d samples at pH 7, 3 replicates

Figure CA. 7.1.1.1.2-4



Section A7.1.1.2.1 **Ready biodegradability**
Annex Point IIA 7.6.1.1

Official use only

1. Reference

1.1	**Reference**	**A7.1.1.2.1/01:** [redacted] Ampholyt 20/100 – Determination of the biodegradability in the DOC die-away test. Infracor GmbH, Marl, Germany, Report No. DDA-179/02, September 25, 2002 (unpublished).
1.2	**Data protection**	Yes
1.2.1	Data owner	Goldschmidt GmbH
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. for the purpose of its entry into Annex I.

2. Guidelines and Quality Assurance

2.1.	**Guideline study**	Yes OECD 301 A, EC method C.4-A
2.2.	**GLP**	Yes
2.3.	**Deviations**	Yes: Test substance concentration, see 0 and 0 unterhalb.

3. Materials and Methods

3.1	**Test material**	Ampholyt 20/100 as given in Section A2.	
3.1.1.	Lot/Batch number	ES62403356	
3.1.2	Specification	Ampholyt 20/100 as given in Section A2. The active substance as manufactured is obtained by the production process as a “product-by-process”, constituting a 20% (w/w) aqueous solution of the active matter. For the purpose of testing physicochemical properties, the material has exceptionally been lyophilised in order to obtain “pure” active substance, termed “Ampholyt 20/100”. Since the a.s. as manufactured consists of 20% active matter and 80% water, the question if lyophilised “Ampholyt 20/100” or the 20% product is subjected to biodegradability testing is considered to be of limited relevance for the reliability of the results.	
3.1.3	Purity	99.4%	X1
	Further relevant properties	The test substance has a stated microbicidal efficacy. To circumvent potential toxic effects on the inoculum, the concentration was limited to 5.17 mg/l DOC. The definition of this figure was based on the result of the bacterial toxicity test (see section A7.4.1.4): EC_{20} = 11.43 mg/l.	X2
3.1.5. Product	Composition of	Not relevant	

3.1.6 TS inhibitory to microorganisms	Yes Result of the bacterial toxicity test (see section A7.4.1.4): EC_{20} = 11.43 mg/l.
3.1.7. Specific chemical analysis	No
3.2. Reference substance	Sodium benzoate Purity > 99.5% FLUKA analysis no.: 364606/161297
3.2.1. Initial concentration of reference substance	16 mg/l (DOC)
3.3. Testing procedure	
3.3.1. Inoculum/ test species	See Table A7.1.1.2.1- 2.
3.3.2. Test system	See Table A7.1.1.2.1- 3.
3.3.3. Test conditions	Test conditions are detailed in Table A7.1.1.2.1- 4.
3.3.4. Method of preparation of test solution	Not relevant; the test substance is highly soluble in water.
3.3.5. Initial TS concentration	5.17 mg/l DOC
3.3.6. Duration of test	28 d
3.3.7. Analytical parameter	Dissolved organic carbon (DOC) concentration
3.3.8. Sampling	0 and 3 hours , then after 7, 14, 21, 27 and 28 days.
3.3.9. Intermediates/ degradation products	Not identified
3.3.3.10. Nitrate/ nitrite measurement	No
3.3.11. Controls	Inoculum blank, toxicity control
3.3.12. Statistics	DOC removal according to test guidelines

4. Results

4.1. Degradation of test substance	
4.1.1 Graph	The graph is given in Figure A7.1.1.2.1- 1 below.
4.1.2 Degradation	> 70% within a 10-d window.
4.1.3Other observations	In the toxicity control, no inhibition of the inoculum was observed.
4.1.4. Degradation of TS in abiotic control	Not performed
4.1.5. Degradation of reference substance	100 % degradation; Also see Figure A7.1.1.2.1- 2.

Section A7.1.1.2.1 Ready biodegradability

Annex Point IIA 7.6.1.1

4.1.6. Intermediates/ degradation products	Not investigated; not necessary.	

Applicant's Summary and conclusion

Materials and methods	The ready biodegradability of the active substance "Ampholyt 20/100" was tested in the DOC die-away test (OECD 301A, EC C.4-A). The performance of the study was compliant to the stated guidelines. Deviating from the guideline, test substance concentration was restricted to only 5.17 mg/l DOC due to its microbicidal properties. The employed concentration was below toxic levels, as also confirmed by the toxicity control.	
Results and discussion	The test substance was degraded by 94% (DOC removal) at the end of the test. The pass level of 70% was reached after 7 days, thus being within the 10-d window. The test substance is not known to exhibit properties having impact on the results.	X3
Conclusion	The validity criteria were fulfilled (see Table A7.1.1.2.1- 5). The test results indicate that the substance is readily biodegradable.	
Reliability	1	
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	14-01-12
Materials and Methods	Applicants version is considered acceptable with the following additions: According to Table A7.1.1.2.1- 3 $MgSO_4$7 H_2O 20.86 mg/L was used to make up the mineral solution. However, the guideline recommends 22.5 g. This deviation from the guideline is considered minor and should not affect the outcome of the experiment. Neither the cell densities nor the amount of effluent (mL effluent/L) of the test solutions were reported. However, the microbial activity of the inoculum appeared to be sufficient according to the degradation of the reference substance. ---------- -- <u>X1</u> Ampholyt 20 consists of 20% active matter and 80 % water. The 20% product was subjected to biodegradability testing. This 20% product was 99.4 % pure in the current test according to the study author. <u>X2</u> The theoretical test item concentration was 5.17 mg DOC/L. The guideline specifies a concentration of 10-40 mg DOC/L as the nominal sole source of organic carbon. The lower concentration was presumably used to prevent toxic effects. According to the *'OECD series on Testing and Assessment Number 27 Guidance Document on the use of the Harmonised System for the Classification of Chemicals which are Hazardous for the Aquatic Environment (23-07-01)* *When it is likely that inhibition is the reason for a substance being not readily degradable, results from a test employing lower non-toxic concentrations of the test substance should be used when available.'* Consequently, the concentration used by the applicant in this study is deemed acceptable in this case. The EC_{20} for the active substance is deemed to be 11 mg/L by the CA ecotoxicology expert.

Results and discussion	Applicant's version is deemed acceptable with the following comments X3 According to the OECD guideline the pass levels for ready biodegradability are 70% removal of DOC in the DOC die-away test. The pass values have to be reached in a 10-d window within the 28-d period of the test. The 10-d window begins when the degree of biodegradation has reached 10% DOC and must end before day 28 of the test. Insufficient measurements were taken in this study to establish when 10% degradation occurred. 3 hr after the initiation of the experiment 0% of the substance had degraded. By day7 72% of the substance had degraded. The study author has calculated the 10 d window based on a very limited number of data points. However, in the current study, the predefined 70% value was exceeded already after 7 days. Therefore, the requirements for a classification as readily biodegradability are fulfilled, and a determination of the 10% value is not considered to be required in this case. Furthermore, Guideline 301A (1992) says: *"Analyse the last samples (28 d) first and, by a stepwise "backwards" selection of appropriate samples for analysis, it is possible to obtain a good description of the biodegradation curve with a relatively small number of determinations", which was explicitly done in these studies.* As a consequence, given the rate of degradation, the low number of measurements should not be considers as a shortcoming. X4 In Table A7.1.1.2.1- 1: the applicant states 60% removal of ThOD or $ThCO_2$ has been met. This criterion does not apply to the test used in this study. In addition ThOD or $ThCO_2$ are not reported in the study report. The percentage degradation of the reference compound (sodium benzoate) reached the pass levels (70 % DOC) by day 14 (e.g. 100 % degradation was observed by 14).
Conclusion	The applicants version is considered acceptable with the following additions: The test substance is not considered inhibitory at 5.17 mg DOC/L as a toxicity test, containing both the test substance and a reference compound, gave >>35% degradation (based on total DOC), **Figure CA 7.1.1.2.1- 1**. According to the test guideline a test is considered inhibitory if < 35 % degradation occurred in a toxicity test. A DOC determination was carried out after 3 hours to determine if there was adsorption of the test substance (test item + inoculum). No significant decrease in DOC occurred after 3 hours. However in the hydrolysis test (IIIA 7.1.1.1.1/02), significant adsorption of Ampholyt 20 to glass was observed after 5 days at pH 7 and 9. The CA therefore considers that the reported degradation may be, in part, due to removal of the test substance by adsorption to glassware, sludge etc. Consequently, the results should be treated with caution. It is concluded the test substance is rapidly *removed* from the test system at the test concentrations tested.
Reliability	The results should be treated with caution. Removal was likely, in part, due to adsorption. However, the study shows the test substance is rapidly removed from the test system
Acceptability	

Remarks	**Post ECHA WG III meeting (Environmental session) 2014** In Study 7.1.1.2.1/01 all validity criteria for the test were satisfied and no significant deviations from test guidelines were reported. The reference substance, sodium benzoate, reached the pass level (70%) by day 14 and thus confirmed the suitability of the inoculum and test conditions. The toxicity control attained 94% degradation after 14 days indicating the lyophilised active substance is non-inhibitory to micro-organisms used in the test. The test substance surpassed the 70% degradation pass level within a 10 day window. A DOC determination was carried out after 3 hours to determine if there was adsorption of the test substance. No significant decrease in DOC occurred after 3 hours. However hydrolysis tests conducted on the active substance indicated significant adsorption of after 5 days at pH 7. Consequently, the reported degradation may be, in part, due to removal of the test substance (e.g. adsorption to glass, sludge). However, it should be noted in the adsorption study (A7.2.3.1/01) the maximum amount adsorbed at adsorption equilibrium was ~reached after 1 hour, whereas during the DOC die-away studies neither adsorption nor degradation was detected in the first 3 hours. In the adsorption study, adsorption to glass was moderate (range 14-18.5% after 24 hours). If a correction for this level adsorption was applied to the ready biodegradability test, Ampholyt 20 would still pass the ready biodegradability criteria. In addition there are several reports in the literature including an ECHA RAC opinion which show that similar substances (amines, coco alkyl, including dodecylamine, and octadecylamine) were ready biodegradable or ready biodegradable with failing the 10-d window.[1] . The CA notes the ECHA RAC opinion stated *'….primary long-chain alkyl amines can be classified as "readily degradable, but failing the 10 d window"* These tests measured carbon dioxide evolution or oxygen consumption. Based on a weight of evidence approach the ECHA WG III meeting (Environmental session) 2014 concluded that Ampholyt 20 is considered as ready biodegradable not fulfilling the 10-day window.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

[1] Committee for Risk Assessment RAC Annex 1 Background document to the Opinion proposing harmonised classification and labelling at Community level of amines, coco alkyl ECHA/RAC/CLH-O-0000002195-77-01/A1 EC number: 262-977-1 CAS number: 61788-46-3, December 2011

Table A7.1.1.2.1- 2: Description of the inoculum.

Criteria	Details
Nature	Activated sludge
Species	Mixed microbial population
Strain	Not applicable
Source	Sewage treatment plant
Sampling site	Municipal STP Marl-Ost, Germany
Laboratory culture	No
Method of cultivation	None
Preparation of inoculum for exposure	Not specified
Pre-treatment	Not specified
Initial cell concentration	Not stated

Table A7.1.1.2.1- 3: Description of the test system.

Criteria	Details
Culturing apparatus	Mechanical shaker
Number of culture flasks/concentration	2 (test substance, inoculum blank) 1 (toxicity control, reference substance)
Aeration device	None
Measuring equipment	Shimadzu T 5000A infrared analyser
Test performed in closed vessels due to significant volatility of test substance	Not required

Table A7.1.1.2.1- 4: Description of the test conditions.

Criteria	Details	
Composition of the medium	KH_2PO_4	85 mg/l
	K_2HPO_4	217.5 mg/l
	$Na_2HPO_4 \cdot 2\ H_20$	334 mg/l
	NH_4Cl	5 mg/l
	$MgSO_4 \cdot 7\ H_20$	20.86 mg/l
	$CaCl_2 \cdot 2\ H_20$	36.4 mg/l
	$FeCl_3 \cdot 2\ H_20$	0.25 mg/l
Additional substrate	None	
Test temperature	22.1°C	
pH	7.4	
Aeration of dilution water	Not stated	
Suspended solids concentration	24.62 mg/l	
Other relevant criteria	None	

Table A7.1.1.2.1- 5: Pass levels and validity criteria for tests on ready biodegradability.

	Fulfilled	Not fulfilled
Pass levels		
60% removal of ThOD or $ThCO_2$	☑	
Pass values reached within 10-d window	☑	
Criteria for validity		
Variation between replicates at the end of test < 20%	☑	
Removal of reference substance reaches pass level by day 14	☑	
Criteria for poorly soluble test substances		
Selection of suitable test method (CO_2 evolution)	n.a.	
Appropriate method of agitation	n.a.	

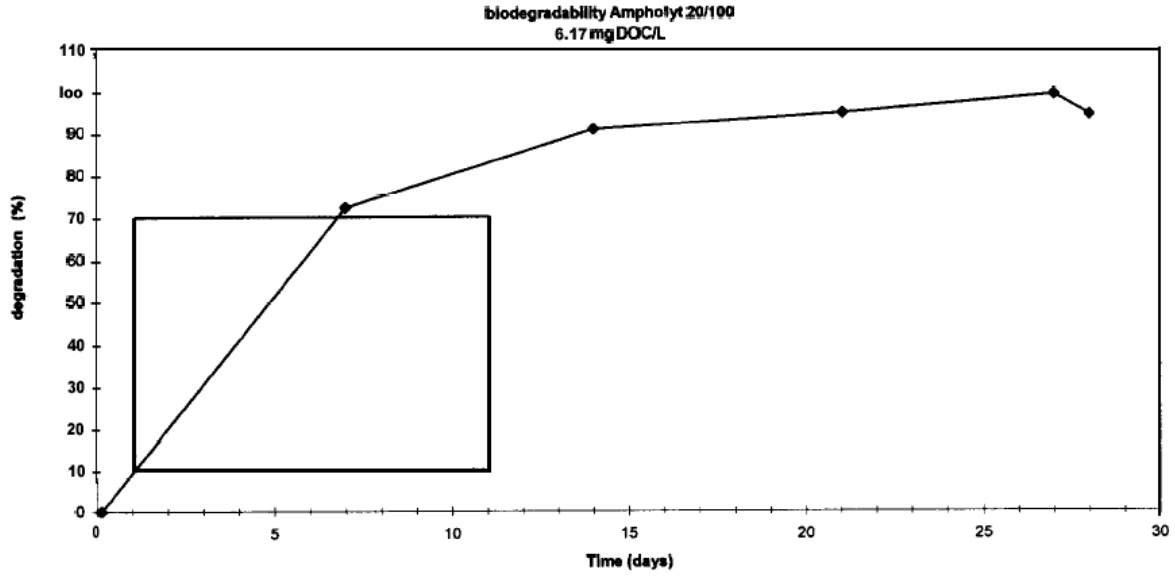


Figure A7.1.1.2.1- 1: Time course of the degradation of Ampholyt 20/100 in the DOC die-away test.

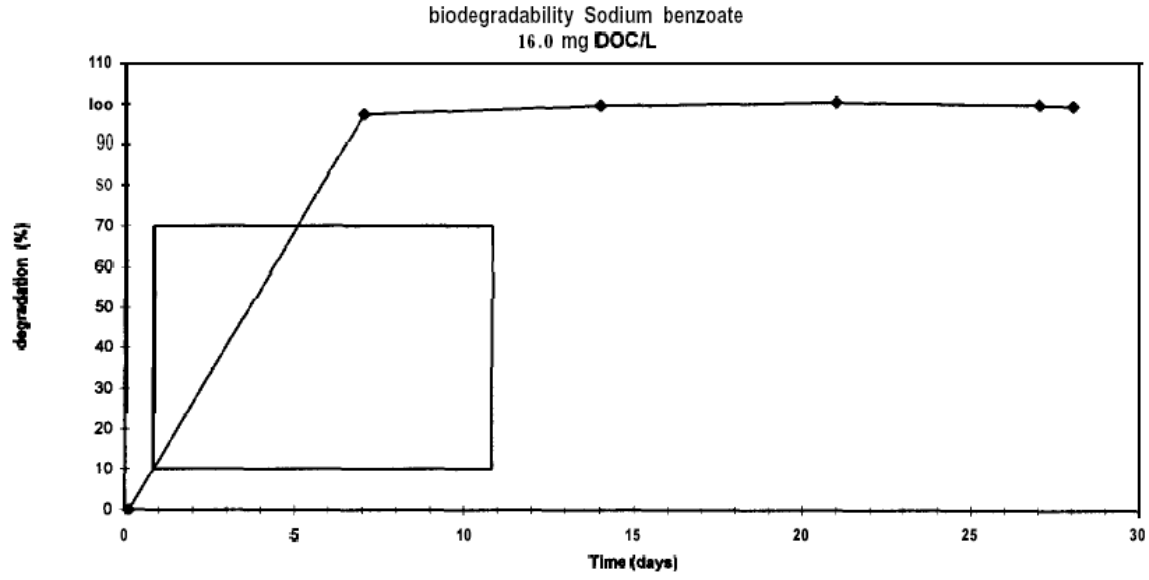


Figure A7.1.1.2.1- 2: Degradation of the reference item (sodium benzoate).

CA data taken from the study report

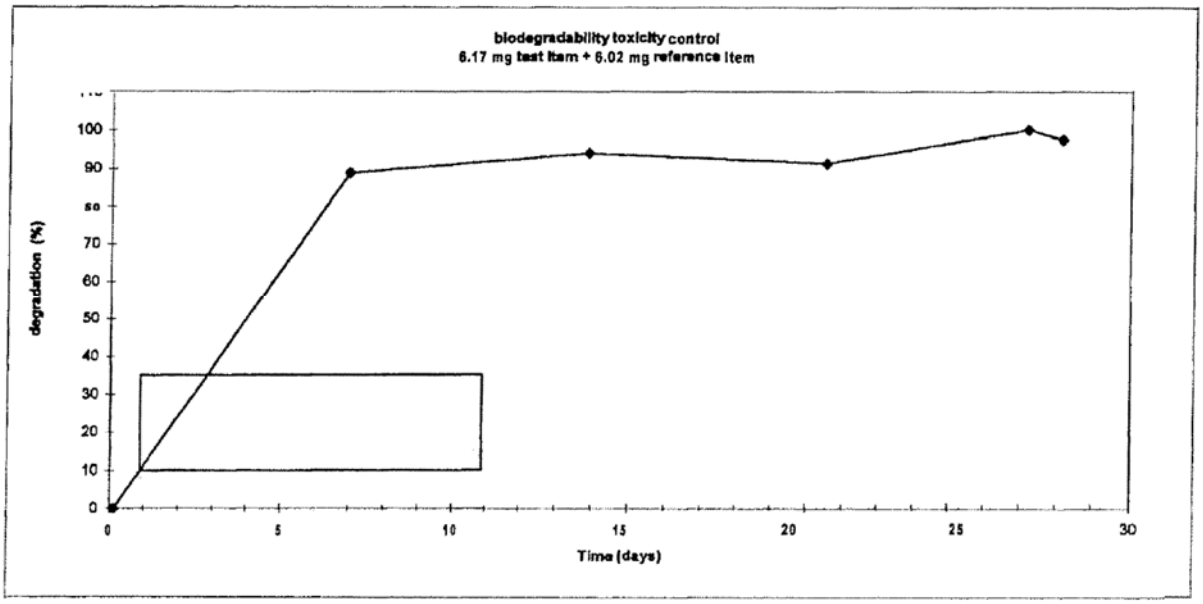


Figure CA 7.1.1.2.1- 1: Toxicity test

Section A7.1.1.2.1 Ready biodegradability

Annex Point IIA 7.6.1.1

Official use only

Reference

Reference	**A7.1.1.2.1/02:** [redacted] TEGO 2000 – Determination of the biodegradability in the DOC die-away test. Infracor GmbH, Marl, Germany, Report No. DDA-163/01, February 05, 2002 (unpublished).
Data protection	Yes
Data owner	Goldschmidt GmbH
Companies with letter of access	No
Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

Guidelines and Quality Assurance

Guideline study	Yes OECD 301 A, EC method C.4-A
GLP	Yes
Deviations	Yes: Test substance concentrations, see 0 and 0 unterhalb.

Materials and Methods

Test material	As given in Section A2. "TEGO 2000" is a synonym (trade name) of the active substance Ampholyt 20, obtained as a "product by process", i.e., a 20% aqueous solution of the pure active.	
Lot/Batch number	17EM17	
Specification	As given in Section A2 for the 20% aqueous solution ("product by process").	
Purity	20% of the pure active in water	
Further relevant properties	The test substance has a stated microbicidal efficacy. To circumvent potential toxic effects on the inoculum, the concentrations were limited to 4.0 and 8.0 mg/l DOC, respectively. The definition of this figure was based on the result of the bacterial toxicity test (see section A7.4.1.4): EC_{20} = 120 mg/l (as regards the 20% concentrated product).	X1
Composition of Product	20% aqueous solution of the active substance "Ampholyt 20/100".	
TS inhibitory to microorganisms	Yes Result of the bacterial toxicity test (see section A7.4.1.4): EC_{20} = 120 mg/l (in terms of the 20% concentrated product). (corresponding to EC_{20} = 24 mg a.i./l)	
Specific chemical analysis	No	

Section A7.1.1.2.1 Ready biodegradability

Annex Point IIA 7.6.1.1

Reference substance	Sodium benzoate Purity > 99.5% FLUKA analysis no.: 364606/161297	
Initial concentration of reference substance	16 mg/l (DOC)	
Testing procedure		
Inoculum/ test species	See Table A7.1.1.2.1- 7.	
Test system	See Table A7.1.1.2.1- 8.	
Test conditions	Test conditions are detailed in Table A7.1.1.2.1- 9.	
Method of preparation of test solution	Not relevant; the test substance is highly soluble in water.	
Initial TS concentration	4.0 and 8.0 mg/l DOC	X1
Duration of test	28 d	
Analytical parameter	DOC concentration	
Sampling	0 and 3 h, then after 7, 14, 21, 27 and 28 days.	
Intermediates/ degradation products	Not identified (not necessary)	
Nitrate/ nitrite measurement	No	
Controls	Inoculum blank, toxicity control	
Statistics	DOC removal according to test guidelines	

Results

Degradation of test substance		
Graph	The graph is given in Figure A7.1.1.2.1- 3 below.	
Degradation	96%, thus > 70% within a 10-day or a 7-day window. 100 % after 28 days.	X2
Other observations	In the toxicity control, no inhibition of the inoculum was observed.	
Degradation of TS in abiotic control	Not performed	
Degradation of reference substance	100% degradation; Also see Figure A7.1.1.2.1- 4.	

Section A7.1.1.2.1 Ready biodegradability

Annex Point IIA 7.6.1.1

Intermediates/ degradation products	Not investigated (not necessary).	

Applicant's Summary and conclusion

Materials and methods	The ready biodegradability of the biocidal product "TEGO 2000", representing a "product by process" in the form of a 20% aqueous solution of the active substance "Ampholyt 20/100" was tested in the DOC die-away test (OECD 301A, EC C.4-A). The performance of the study was compliant to the stated guidelines. Deviating from the guideline, initial test item concentrations were only 4.2 and 8.2 mg/l DOC, respectively, due to the microbicidal properties of the product. The employed concentrations were below toxic levels, as also confirmed by the toxicity control.	
Results and discussion	The test substance was degraded by 96% (DOC removal) at the end of the test. The pass level of 70% was reached after 7 days, thus being within the 10-d window. The test substance is not known to exhibit properties having impact on the results.	X2
Conclusion	The validity criteria were fulfilled (see Table A7.1.1.2.1- 10). The test results indicate that the substance is readily biodegradable.	X3
Reliability	1	
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	14-01-13
Materials and Methods	Applicants version is considered acceptable with the following additions: X1 The theoretical test item concentrations were 4.2 mg DOC/L and 8.2 mg DOC/L. The guideline specifies a concentration of 10-40 mg DOC/L as the nominal sole source of organic carbon. The lower concentrations were presumably used to prevent toxic effects. According to the *'OECD series on Testing and Assessment Number 27 Guidance Document on the use of the Harmonised System for the Classification of Chemicals which are Hazardous for the Aquatic Environment (23-07-01)* *When it is likely that inhibition is the reason for a substance being not readily degradable, results from a test employing lower non-toxic concentrations of the test substance should be used when available.'* Consequently, the concentration used by the applicant in this study is deemed acceptable in this case. Neither the cell densities nor the amount of effluent (mL effluent/L) of the test solutions were reported. Although the microbial activity of the inoculum appeared to be sufficient according to the degradation of the reference substance.

Results and discussion	The applicants version is considered acceptable with the following additions: X2 According to the OECD guideline the pass levels for ready biodegradability are 70% removal of DOC in the DOC die-away test. The pass values have to be reached in a 10-d window within the 28-d period of the test. The 10-d window begins when the degree of biodegradation has reached 10% DOC and must end before day 28 of the test. Insufficient measurements were taken in this study to establish when 10% degradation occurred. 3 hr after the initiation of the experiment 0% of the substance had degraded. By day 7 64.64-92.01 % of the substance had degraded when the test substance concentration was 4.2 mg DOC/L. The corresponding value for 6.2 mg DOC/L (t = 0 d) was 82.85-86.31 %. The study author has calculated the 10 d window based on a very limited number of data points. Consequently, there is uncertainty in the results. However, the predefined 70% value was exceeded already after 7 days. Therefore, the requirements for a classification as readily biodegradability are fulfilled, and a determination of the 10% value is not considered to be required in this case. Furthermore, Guideline 301A (1992) says: *"Analyse the last samples (28 d) first and, by a stepwise "backwards" selection of appropriate samples for analysis, it is possible to obtain a good description of the biodegradation curve with a relatively small number of determinations"*, which was explicitly done in these studies. As a consequence, given the rate of degradation, the low number of measurements should not be considers as a shortcoming. X3 In Table A7.1.1.2.1- 6 the applicant states the 60% removal of ThOD or $ThCO_2$ has been met. This criterion does not apply to the test used in this study. In addition ThOD or $ThCO_2$ are not reported in the study report. The percentage degradation of the reference compound (sodium benzoate) reached the pass levels (70 % DOC) by day 14 (e.g. 97 % degradation observed by 14). The test substance is not considered inhibitory between 4.2-8.2 mg DOC/L as a toxicity test, containing both the test substance and a reference compound, gave >35% degradation (based on total DOC). According to the test guideline a test is considered inhibitory if < 35 % degradation occurred in a toxicity test.
Conclusion	The applicants version is considered acceptable with the following additions: A DOC determination was carried out after 3 hours to determine if there was adsorption of the test substance (test item + inoculum). No significant decrease in DOC occurred after 3 hours. However in the hydrolysis test (IIIA 7.1.1.1.1/02), significant adsorption of Ampholyt 20 to glass was observed after 5 days at pH 7 and 9. The CA therefore considers that the reported degradation may be, in part, due to removal of the test substance by adsorption to glassware, sludge etc. Consequently, the results should be treated with caution. It is concluded the test substance is rapidly *removed* from the test system at the test concentrations tested
Reliability	2
Acceptability	The results should be treated with caution. Removal was likely, in part, due to adsorption. However, the study shows the test substance is rapidly removed from the test system.

Remarks	**Post ECHA WG III meeting (Environmental session) 2014** The percentage degradation of the reference compound (sodium benzoate) reached the pass levels (70 % DOC) by day 14 (e.g. 97 % degradation observed by 14). The test substance is not considered inhibitory between 4.2-8.2 mg DOC/L as a toxicity test, containing both the test substance and a reference compound, gave >35% degradation (based on total DOC. The removal of the test substance was 96% (DOC removal) at the end of the test. The pass level of 70% was reached after 7 days, thus being within the 10-d window. A DOC determination was carried out after 3 hours to determine if there was adsorption of the test substance. No significant decrease in DOC occurred after 3 hours. However hydrolysis tests conducted on the active substance indicated significant adsorption of after 5 days at pH 7. Consequently, the reported degradation may be, in part, due to removal of the test substance (e.g. adsorption to glass, sludge). However, it should be noted in the adsorption study (A7.2.3.1/01) the maximum amount adsorbed at adsorption equilibrium was ~reached after 1 hour, whereas during the DOC die-away studies neither adsorption nor degradation was detected in the first 3 hours. In the adsorption study, adsorption to glass was moderate (range 14-18.5% after 24 hours). If a correction for this level adsorption was applied to the ready biodegradability test, Ampholyt 20 would still pass the ready biodegradability criteria. In addition there are several reports in the literature including an ECHA RAC opinion which show that similar substances (amines, coco alkyl, including dodecylamine, and octadecylamine) were ready biodegradable or ready biodegradable with failing the 10-d window.[2] . The CA notes the ECHA RAC opinion stated *'….primary long-chain alkyl amines can be classified as "readily degradable, but failing the 10 d window"* These tests measured carbon dioxide evolution or oxygen consumption. Based on a weight of evidence approach the ECHA WG III meeting (Environmental session) 2014 concluded that Ampholyt 20 is considered as ready biodegradable not fulfilling the 10-day window.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

[2] Committee for Risk Assessment RAC Annex 1 Background document to the Opinion proposing harmonised classification and labelling at Community level of amines, coco alkyl ECHA/RAC/CLH-O-0000002195-77-01/A1 EC number: 262-977-1 CAS number: 61788-46-3, December 2011

Table A7.1.1.2.1- 7: Description of the inoculum.

Criteria	Details
Nature	Activated sludge
Species	Mixed microbial population
Strain	Not applicable
Source	Sewage treatment plant
Sampling site	Municipal STP Marl-Ost, Germany
Laboratory culture	No
Method of cultivation	None
Preparation of inoculum for exposure	Not specified
Pre-treatment	Not specified
Initial cell concentration	Not stated

Table A7.1.1.2.1- 8: Description of the test system.

Criteria	Details
Culturing apparatus	Mechanical shaker
Number of culture flasks/concentration	2 (test substance, inoculum blank) 1 (toxicity control, reference substance)
Aeration device	None
Measuring equipment	Shimadzu T 500 infrared analyser
Test performed in closed vessels due to significant volatility of test substance	Not required

Table A7.1.1.2.1- 9: Description of the test conditions.

Criteria	Details	
Composition of the medium	KH_2PO_4	85 mg/l
	K_2HPO_4	217.5 mg/l
	$Na_2HPO_4 \cdot 2\ H_20$	334 mg/l
	NH_4Cl	5 mg/l
	$MgSO_4 \cdot 7\ H_20$	22.5 mg/l
	$CaCl_2 \cdot 2\ H_20$	36.4 mg/l
	$FeCl_3 \cdot 2\ H_20$	0.25 mg/l
Additional substrate	None	
Test temperature	21.7 °C (range: 21.0–23.0 °C)	
pH	7.4	
Aeration of dilution water	Not stated	
Suspended solids concentration	25.86 mg/l	
Other relevant criteria	None	

Table A7.1.1.2.1- 10: Pass levels and validity criteria for tests on ready biodegradability.

	Fulfilled	Not fulfilled
Pass levels		
60% removal of ThOD or $ThCO_2$	☑	
Pass values reached within 10-d window	☑	
Criteria for validity		
Variation between replicates at the end of test < 20%	☑	
Removal of reference substance reaches pass level by day 14	☑	
Criteria for poorly soluble test substances		
Selection of suitable test method (CO_2 evolution)	n.a.	
Appropriate method of agitation	n.a.	

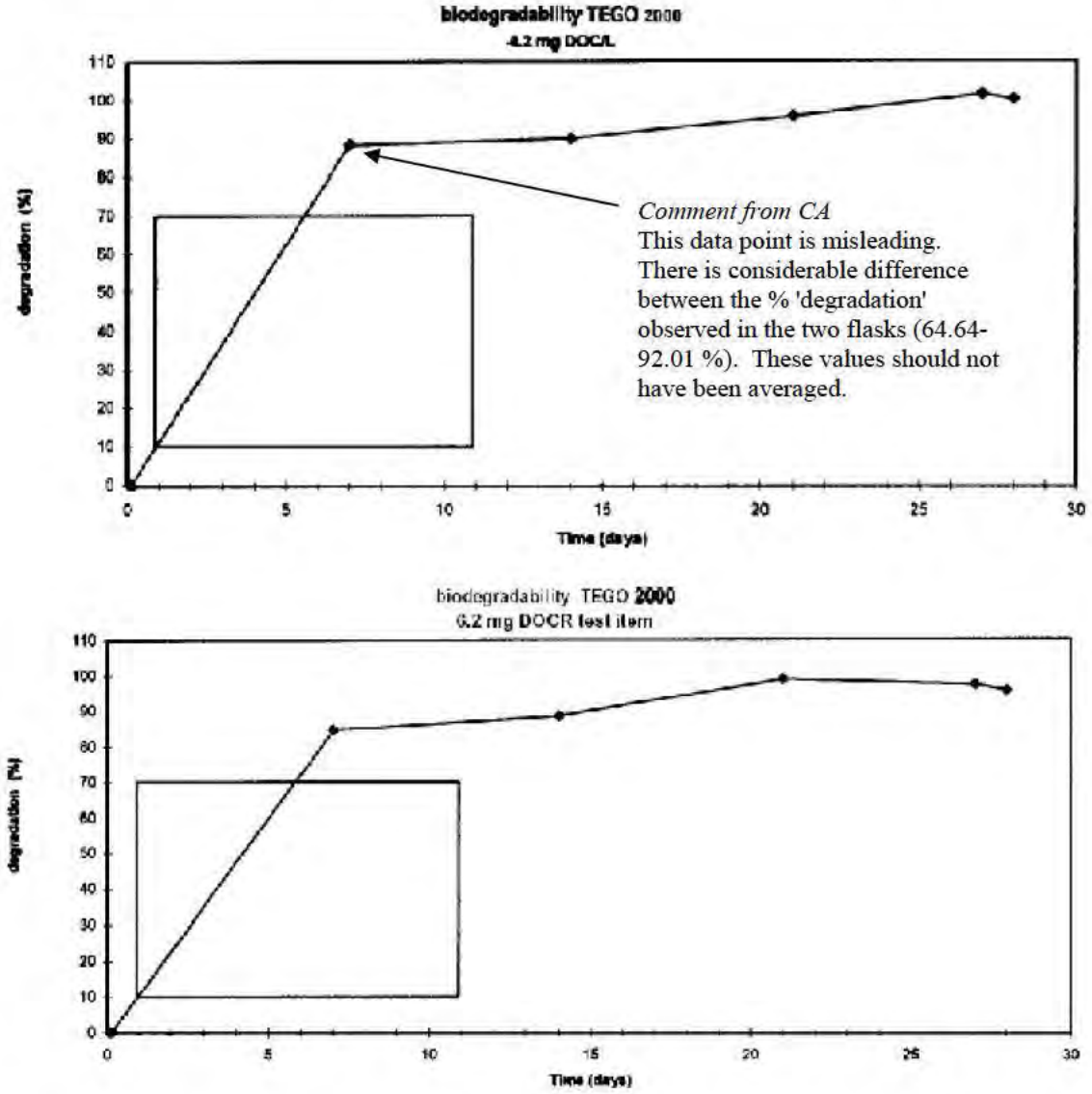


Figure A7.1.1.2.1- 3: Time course of the degradation of TEGO 2000 in the DOC die-away test at two different concentrations; the stated concentration in the lower sub-figure is a typing error – the correct value is 8.2 mg/l.

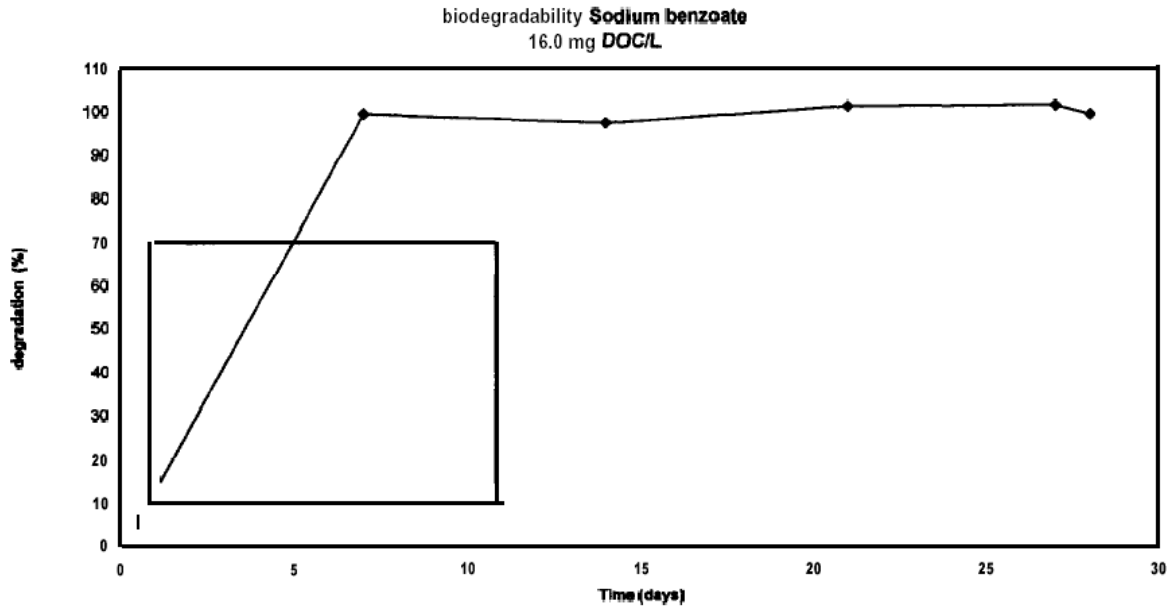


Figure A7.1.1.2.1- 4: Degradation of the reference item (sodium benzoate).

Section A7.1.1.2.1 Ready biodegradability

Annex Point IIA 7.6.1.1

		Official use only
	Reference	
1.1. Reference	**A7.1.1.2.1/03:** [redacted] (1991) The biodegradability of the product TEGO®2000/TEGOL®2000 in a closed bottle test according to a draft OECD guideline: ready biodegradability. TNO, Delft, The Netherlands, Report No. R91/221, September 03, 1991 (unpublished).	
1.2. Data protection	Yes	
1.2.1.Data owner	Degussa-Goldschmidt	
1.2.2.Companies with letter of access	No	
1.2.3Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
	2. Guidelines and Quality Assurance	
2.1. Guideline study	Yes Draft OECD guideline on ready biodegradability, which is consistent to the currently adopted OECD guideline 301 and EC method C.4 in all important aspects.	
2.2. GLP	Yes	
2.3.Deviations	Yes: Test duration, see 0 unterhalb; Cell densities or suspended solid concentrations in the test solutions were not reported (Table A7.1.1.2.1- 11, Table A7.1.1.2.1- 12)	
	3. Materials and Methods	
3.1Test material	As given in Section A2. "TEGO 2000" is a synonym (trade name) of the active substance Ampholyt 20, obtained as a "product by process", i.e., a 20% aqueous solution of the pure active.	
3.1.1 Lot/Batch number	1130199	
3.1.2. Specification	As given in Section A2 for the 20% aqueous solution ("product by process").	
3.1.3 Purity	20% of the pure active in water	
3.1.4 Further relevant properties	The test substance has a stated microbicidal efficacy. To circumvent potential toxic effects on the inoculum, the concentrations were limited to 2.02 and 4.04 mg/l DOC, respectively. The definition of this figure was based on the result of the bacterial toxicity test (see section A7.4.1.4): EC_{20} = 120 mg/l (in terms of the 20% concentrated product).	X1
3.1.5 Composition of Product	20% aqueous solution of the active substance "Ampholyt 20/100".	

Section A7.1.1.2.1 Ready biodegradability

Annex Point IIA 7.6.1.1

3.1.6. TS inhibitory to microorganisms	Yes Result of the bacterial toxicity test (see section A7.4.1.4): EC_{20} = 11.43 mg/l.	
3.1.7. Specific chemical analysis	No	
3.2. Reference substance	Sodium acetate	
3.2.1. Initial concentration of reference substance	4.01 mg/l	
3.3. Testing procedure		
3.3.1.Inoculum/ test species	See Table A7.1.1.2.1- 11.	
3.3.2. Test system	See Table A7.1.1.2.1- 12.	
3.3.3. Test conditions	Test conditions are detailed in Table A7.1.1.2.1- 13.	
3.3.4 Method of preparation of test solution	Not relevant; the test substance is highly soluble in water.	
3.3.5. Initial TS concentration	2.02 and 4.04 mg/l	
3.3.6. Duration of test	29 d	
3.3.7. Analytical parameter	Oxygen concentration	
3.3.8 Sampling	At the start of the test (1 bottle per group), and after 7, 14, 21 and 29 days.	
3.3.9. Intermediates/ degradation products	Not identified	
3.3.10 Nitrate/ nitrite measurement	No	
3.3.11 Controls	Inoculum blank, toxicity control	
3.312. Statistics	Oxygen consumption according to test guidelines.	

4. Results

4.1.1 Degradation of test substance		
4.1.2 Graph	A graph of oxygen consumption in the various test groups is given in Figure A7.1.1.2.1- 1 below. For the percent degradation, no graph is available from the study report. Alternatively, degradation is presented in tabular form in Table A7.1.1.2.1- 14.	X2
4.1.3. Degradation	Since there is no plateau phase, no degradation level can be given. At an initial TS concentration of 2.02 mg/l, 30% degradation was reached after 14 days, and then dropped to 6% and 11% on days 21 and 29, respectively (also see Table A7.1.1.2.1- 14).	
4.1.4. Other observations	In the toxicity control, the test substance proved to be inhibitory to the inoculum.	
4.1.5 Degradation of TS in abiotic control	Not performed	

Section A7.1.1.2.1 Ready biodegradability

Annex Point IIA 7.6.1.1

4.1.6 Degradation of reference substance	Complete degradation within 14 days.	
4.1.7. Intermediates/ degradation products	Not investigated.	

5. Applicant's Summary and conclusion

5.1. Materials and methods	The ready biodegradability of the biocidal product TEGO 2000, constituting a "product by process" containing the active substance "Ampholyt 20/100", was tested in a closed bottle test (prior to adoption of international guidelines but consistent to OECD 301D and EC C.4-E in all important aspects). The test was inadvertently performed over 29 days, but this deviation is not considered to have an impact on the results. A potential influence of nitrification was not considered. Neither cell densities nor suspended solid matter concentration in the test solutions were reported. Although the microbial activity of the inoculum appeared to be sufficient according to the degradation of the reference substance, the lack of information on cell densities limits the reliability of the study.	X3
5.2. Results and discussion	The test substance is known to exhibit microbicidal properties. In fact, the results of the toxicity control indicate that the inoculum may have been inhibited by the test item to a varying degree. The test failed the pass levels for ready biodegradability at any sampling time. However, the inconsistent time course of percent degradation (Table A7.1.1.2.1- 14) indicates problems with the stability of the test system and the repeatability of the results under the employed test conditions.	
5.3. Conclusion	The validity criteria were formally fulfilled (see Table A7.1.1.2.1- 15). However, the various deficiencies and problems discussed within this chapter lead to the conclusion that the study should not be regarded as valid.	
5.3.1. Reliability	3	
5.3.2. Deficiencies	Yes: The methodological deficiencies as discussed in 0 oben, along with the problems associated with the temporal variation of degradation (see 0 oben), severely limit the validity of this 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	15/01/13
Materials and Methods	The Applicants version is deemed acceptable with the following comments: The amount of disodium hydrogen orthophosphate dihydrate used in the mineral medium is higher than what is proposed in the guideline (50.30 g Vs 33.4 g) The exact proportions of the major components of the test substance are unknown. Consequently, it is not possible to calculate the ThOD. In these circumstances the guideline recommends calculating the COD. Degradation was calculated as BOD/COD x 100/1 in this study. The guidance document notes this is less satisfactory relative to the corrected BOD/ThOD x 100/1. The day 0 oxygen measurement ranged from 9.3 to 9.5 mg/L. According to the guideline the the concentration of dissolved oxygen for purposes should be about 9 mg/L at 20°C. This suggests the mineral medium was fully aerated. The notifier notes cell densities were not reported. The CA notes the guideline states *'In order to ensure that the inoculum activity is not limited, the concentration of dissolved oxygen must not fall below 0.5 mg/l in the BOD bottles. This limits the concentration of test substance in general.'* The CA notes the concentration of dissolved oxygen is above the data requirement for the test substance and control samples. <u>X1</u> The test concentrations were 2.02 and 4.01 mg/L not 2.02 mg DOC/L and 4.01 mg DOC/L. These are in the recommended range prescribed by the guideline (2-5 mg/L). <u>X2</u> CA has presented a graph of the percent degradation in Figure CA7.1.1.2.1.03-1 Since there is no plateau phase, no degradation level can be given. At an initial TS concentration of 2.02 mg/l, 30% degradation was reached after 14 days, and then dropped to 6% and 11% on days 21 and 29, respectively <u>X3</u> As pointed out by the applicant, nitrification was not considered. Consequently, the reported degradation rates are likely to be overestimated. OECD guidance states *'for test substances containing N, serious errors can arise if the observed oxygen uptake is not corrected for the amount of oxygen used in oxidising ammonium to nitrite and nitrate'*

Results and discussion

The Applicants version is considered acceptable with the following additions:

The study is considered to meet the validity criteria specified:

Validity criteria	Fulfilled	Unfulfilled
Oxygen depletion in the inoculum blank should not exceed 1.5 mg dissolved oxygen/l after 28 days	X	
The residual concentration of oxygen in the test bottles should not fall below 0.5 mg/L at any time.	X	
A test is considered valid if the difference of extremes of replicate values of the removal of the test chemical at the plateau, at the end of the test or at the end of the 10-d window, as appropriate, is less than 20% **and** if the percentage degradation of the reference compound has reached the pass levels by day 14.	X* X	

* plateau was not reached

The measured oxygen consumption of acetate after 14 d was 0.61 mg O_2/mg. This compares well with the ThOD of 0.68 mg O_2/mg. In the presence of the test substance the oxygen consumption rate for acetate was 0.53 and 0.49 mg O_2/mg (day 14) at test concentrations of 2.04 and 4.08 mg test substance/L respectively. This suggests some inhibition of the test substance.

Conclusion

Applicants version is deemed acceptable with the following comment:
Ampholyt 20 is not considered biodegradable under the conditions of the study.

Reliability

3

Acceptability

The study is not considered suitable for risk assessment due the reasons outlined in Sections 5.1 and 5.2. However, the study provides some useful information. The study suggests the test substance may be toxic to microorganisms at certain test concentrations. This is to be expected as Ampholyt 20 has antimicrobial properties.

Remarks

COMMENTS FROM ...

Date

Materials and Methods

Results and discussion

Conclusion

Reliability

Acceptability

Remarks

Table A7.1.1.2.1- 11: Description of the inoculum.

Criteria	Details
Nature	Activated sludge
Species	Mixed microbial population
Strain	Not applicable
Source	Oxidation ditch at the performing laboratory
Sampling site	TNO, Delft, The Netherlands
Laboratory culture	No
Method of cultivation	None
Preparation of inoculum for exposure	Settling for 2 min, supernatant taken for inoculation
Pre-treatment	Not specified
Initial cell concentration	Not stated

Table A7.1.1.2.1- 12: Description of the test system.

Criteria	Details
Culturing apparatus	Not stated
Number of culture flasks/concentration	3
Aeration device	None
Measuring equipment	Oxygen electrode
Test performed in closed vessels due to significant volatility of test substance	Not required

Table A7.1.1.2.1- 13: Description of the test conditions.

Criteria	Details		
Composition of the medium	Stock solutions as follows:		
	a)	KH_2PO_4	8.5 g/l
		K_2HPO_4	21.75 g/l
		$Na_2HPO_4 \cdot 7\ H_20$	50.3 g/l
		NH_4Cl	0.5 g/l
	b)	$MgSO_4 \cdot 7\ H_20$	22.5 g/l
	c)	$CaCl_2$	27.5 g/l
	d)	$FeCl_3 \cdot 2\ H_20$	0.2 g/l
	Final concentrations in the mineral medium were not specified.		
Additional substrate	None		
Test temperature	Ca. 20°C		
pH	7.3		
Aeration of dilution water	Vigorous aeration before use		
Suspended solids concentration	4.3 g/l (prior to settling)		
Other relevant criteria	None		

Table A7.1.1.2.1- 14: Biodegradation of the test substance, as % of COD.

	2.02 mg/1		4.04 mg/1	
Time (days)	**mg O_2/mg TS**	**% biodegradation**	**mg O_2/mg TS**	**% biodegradation**
7	0.08	17		
14	0.14	30		
21	0.03	6	0.05	11
29	0.05	11	0.04	9

Table A7.1.1.2.1- 15: Pass levels and validity criteria for tests on ready biodegradability.

	Fulfilled	Not fulfilled
Pass levels		
60% removal of ThOD or $ThCO_2$		☑
Pass values reached within 10-d window		☑
Criteria for validity		
Variation between replicates at the end of test < 20%	☑	
Removal of reference substance reaches pass level by day 14	☑	
Criteria for poorly soluble test substances		
Selection of suitable test method (CO_2 evolution)	n.a.	
Appropriate method of agitation	n.a.	

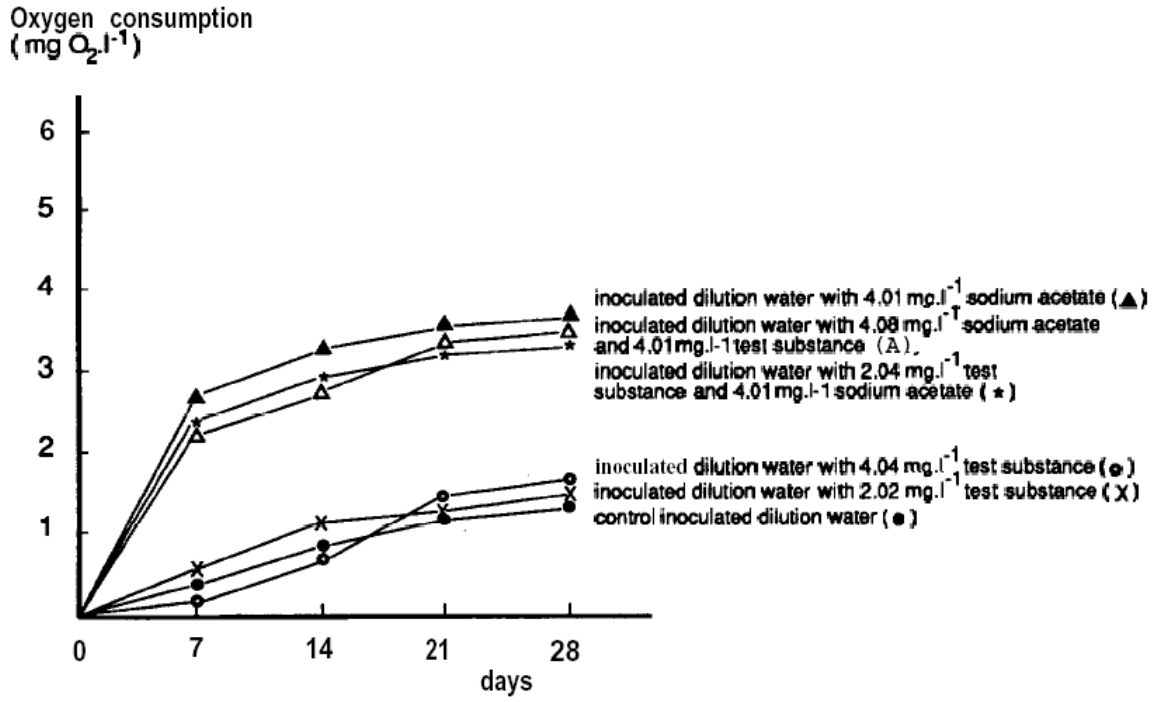


Figure A7.1.1.2.1- 5: Oxygen consumption in various groups of the closed bottle test on TEGO 2000.

Data taken from Study report by the CA

Oxygen concentrations in the control test and calculated BOD values (standard deviations in brackets)

Conc. TEGO® 2000/ TEGOL® 2000 mg.l^{-1}	0 days	7 days		14 days		21 days		29 days	
	O_2 conc. mg.l^{-1}	O_2 conc. mg.l^{-1}	BOD7 [a] mg.l^{-1} mg.mg^{-1}	O_2 conc. mg.l^{-1}	BOD14 [a] mg.l^{-1} mg.mg^{-1}	O_2 conc. mg.l^{-1}	BOD21 [a] mg.l^{-1} mg.mg^{-1}	O_2 conc. mg.l^{-1}	BOD29 [a] mg.l^{-1} mg.mg^{-1}
0	9.4	9.03 (0.15)	0.37	8.55 (0.07)	0.85	8.23 (0.06)	1.17	8.13 (0.06)	1.27
2.02	9.5	8.97 (0.12)	0.53 0.08[b]	8.37 (0.06)	1.13 0.14[b]	8.27 (0.15)	1.23 0.03[b]	8.13 (0.06)	1.37 0.05[b]
4.04	9.3	9.07 (0.06)	0.23 –[b]	8.57 (0.06)	0.73 –[b]	7.93 (0.12)	1.37 0.05[b]	7.87 (0.06)	1.43 0.04[b]

a) Oxygen consumption in the indicated period.
b) BOD attributable to the test substance.
* Two of the three bottles used for calculation.

Oxygen concentrations in the biodegradation test and calculated BOD values (standard deviations in brackets)

Conc. TEGO® 2000/ TEGOL® 2000 mg.l^{-1}	0 days	7 days		14 days		21 days		29 days	
	O_2 conc. mg.l^{-1}	O_2 conc. mg.l^{-1}	BOD7 [a] mg.l^{-1} mg.mg^{-1}	O_2 conc. mg.l^{-1}	BOD14 [a] mg.l^{-1} mg.mg^{-1}	O_2 conc. mg.l^{-1}	BOD21 [a] mg.l^{-1} mg.mg^{-1}	O_2 conc. mg.l^{-1}	BOD29 [a] mg.l^{-1} mg.mg^{-1}
0	9.4	9.03 (0.15)	0.37	8.55 (0.07)	0.85	8.23 (0.06)	1.17	8.13 (0.06)	1.27
2.02	9.5	8.97 (0.12)	0.53 0.08[b]	8.37 (0.06)	1.13 0.14[b]	8.27 (0.15)	1.23 0.03[b]	8.13 (0.06)	1.37 0.05[b]
4.04	9.3	9.07 (0.06)	0.23 –[b]	8.57 (0.06)	0.73 –[b]	7.93 (0.12)	1.37 0.05[b]	7.87 (0.06)	1.43 0.04[b]

a) Oxygen consumption in the indicated period.
b) BOD attributable to the test substance.
* Two of the three bottles used for calculation.

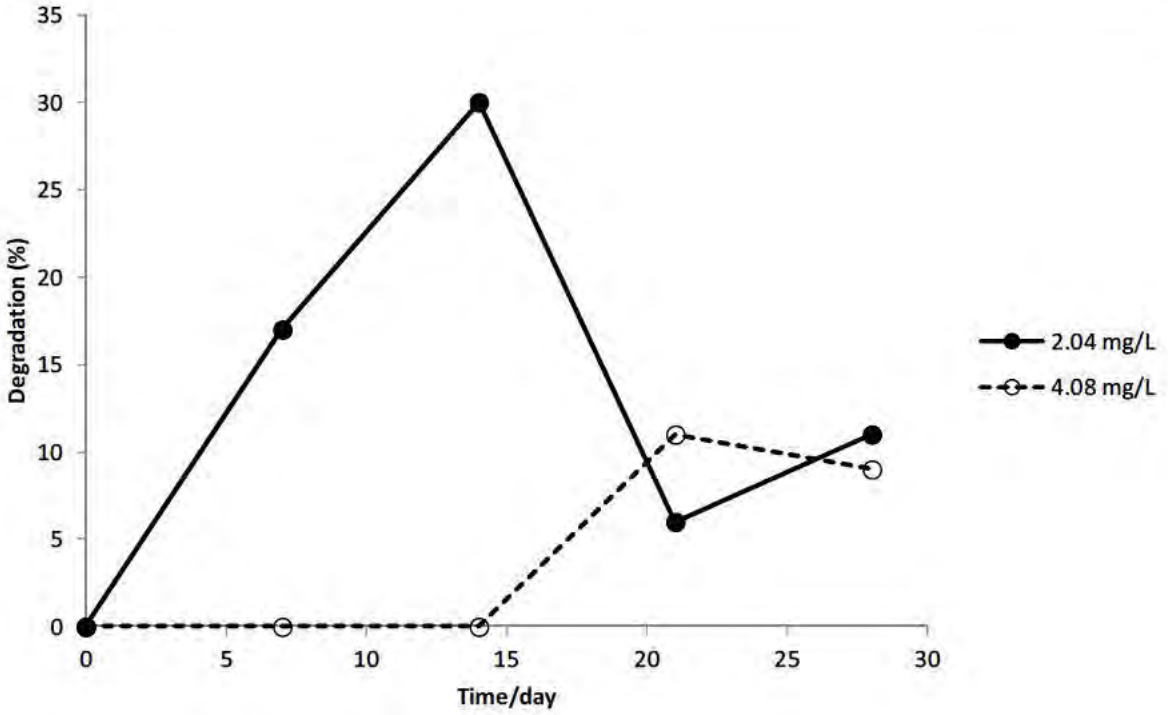


Figure CA7.1.1.2.1.03-1: Degradation of the test substance

Section A7.1.1.2.2 Inherent biodegradability

Annex Point IIA 7.6.1.2

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	The ready biodegradability of Ampholyt 20 has been demonstrated in Section A7.1.1.2.1. Therefore, no further studies are required since the established "ready biodegradability" and the resulting conclusions for risk assessment extend beyond results potentially to be derived from inherent biodegradability. Further, based on the decision tree in subchapter 7.0.2 (Testing Strategy on biodegradation of biocidal active substances), the assessment of rate constants is already possible in the case that the criterion for ready biodegradability is fulfilled, and further testing is not explicitly recommended.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	16/01/13
Evaluation of applicant's justification	Applicant's justification is considered acceptable
Conclusion	An Inherent biodegradability study is considered a core data requirement when a compound is not readily degradable unless a simulation test is performed. Ampholyt 20/Ampholyt 20/100 was rapidly removed from the test systems used to investigate ready biodegradability (Studies 7.1.1.2.1/01 &7.1.1.2.1/02). The CA considers that the reported degradation may be, in part, due to removal of the test substance by adsorption to glassware, sludge etc. Consequently, the results should be treated with caution. In view of the rapid *removal* of the test substance from these systems a study on inherent biodegradability is not considered required.
Remarks	
	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A7.1.1.2.3 Biodegradation in seawater

Annex Point IIIA 12.2.1

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	According to chapter 3 the TNsG on data requirements, section 7.1.1.2.3, biodegradation testing in seawater is only required if a substance is to be used or released in marine environments in considerable amounts (e.g. it is known to be repeatedly used or continuously released in marine environments). Only in such cases a seawater biodegradation test according to OECD guideline 306 will be required. However, with respect to the field of use envisaged, release of the product and the active substance to seawater can be safely excluded. The application as a disinfectant (microbicidal agent for surfaces) does not entail either repeated/continuous or even unique release to the marine environment.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	16/01/13	
Evaluation of applicant's justification	Applicant's justification is considered acceptable	
Conclusion	The release of the product and the active substance to seawater can be safely excluded. The application as a disinfectant (microbicidal agent for surfaces) does not entail either repeated/continuous or even unique release to the marine environment.	
Remarks		
	COMMENTS FROM ...	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A7.1.2.1.1 **Biological sewage treatment: Aerobic biodegradation**
Annex Point IIIA 11.2.1

Official use only

1. Reference

1.1	**Reference**	**A7.1.2.1.1/01:** [redacted] (1993) The elimination of TEGO®2000/TEGOL®2000 in a continuous activated sludge system ("Coupled Units Test"). TNO, Delft, The Netherlands, Report No. IMW-R92/327, October 05, 1993 (unpublished).
1.2	**Data protection**	Yes
1.3	Data owner	Goldschmidt GmbH
1.4	Companies with letter of access	No
1.5	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

2. Guidelines and Quality Assurance

2.1	**Guideline study**	Yes OECD 303A
2.2	**GLP**	Yes
2.3.	**Deviations**	Yes The activated sludge was not a composite inoculum, as required by the guideline, but from only a single source (see 0 unterhalb, Table A7.1.2.1.1- 1); however, the impact of this deviation on the reliability of the study is considered to be negligible. The synthetic sewage stock solution was fourfold concentrated compared to the guideline instructions (0, Table A7.1.1.2.1- 4); since this was compensated via the dosing rate, there was no impact on the study resulting from this deviation. Removal of particulate material from the effluent by centrifugation (see 0 unterhalb) instead of membrane filtration; the effect on the reliability of the study is considered to be negligible.

3 Materials and Methods

3.1	**Test material**	As given in Section A2. "TEGO 2000" is a trade name of the active substance Ampholyt 20, obtained as a "product by process", i.e., a 20% aqueous solution of the pure active.
3.2	Lot/Batch number	1450245
3.3	Specification	As given in Section A2 for the 20% aqueous solution ("product by process").
3.4	Purity	20% of the pure active in water
3.5.	Further relevant properties	The test substance has a claimed microbicidal efficacy (see 0 unterhalb).
3.6	Composition of Product	20% aqueous solution of the active substance "Ampholyt 20/100".

Section A7.1.2.1.1 Biological sewage treatment: Aerobic biodegradation

Annex Point IIIA 11.2.1

3.7 TS inhibitory to microorganisms	Yes Result of the bacterial toxicity test (see section A7.4.1.4): EC_{20} = 120 mg/l (in terms of the 20% concentrated product).	
3.8 Specific chemical analysis	No	
3.9 Reference substance	None	
3.10 Initial concentration of reference substance		
3.11 Testing procedure		
3.12 Inoculum/ test species	Details are given in Table A7.1.2.1.1- 1.	
3.13 Test system	See Table A7.1.1.2.1- 3.	
3.14 Test conditions	Test conditions are described in Table A7.1.1.2.1- 4.	
3.15 Method of preparation of test solution	Not relevant; the test substance is highly soluble in water.	
3.16 Initial TS concentration	120 mg DOC/l (mean)	
3.17 Duration of test	25 days	
3.18 Analytical parameter	DOC (after centrifugation at g_{max} of 2000 for removal particulate material)	
3.19 Sampling	Daily	
3.20 Intermediates/ degradation products	Not identified	
3.21 Nitrate/ nitrite measurement	Not applicable	
3.22 Controls	Blank unit	
3.23 Statistics	Degradation rate according to test guideline.	

4. Results

4.1 Degradation of test substance		
4.2 Graph	Not applicable for this test. However, please refer to Figure A7.1.2.1.1-2	
4.3 Degradation	The results indicate a nearly complete removal of the test substance in the coupled unit test. The plateau level was reached in 8 days and the mean degradation percentage was calculated from 10 plateau values to be 81 ± 5%. *DR* = 81 ± 5% (mean ± SD)	X1
4.4 Other observations	None	
4.5 Degradation of TS in abiotic control	Not applicable for this test.	
4.6 Degradation of reference substance	Not applicable for this test.	

4.7 Intermediates/ degradation products	None

5. Applicant's Summary and conclusion

5.1 Materials and methods	The degradation of the active substance of TEGO 2000 (Ampholyt 20/100) was simulated in a coupled units test (OECD 303A). The performance of the test deviated from the guideline in several aspects, as discussed under 0 oben. The fact that the inoculum originated from only a single source is considered to be without negative consequences since the microbial activity proved to be adequate. Deviations in the concentration of the synthetic sewage were compensated during dosing. Centrifugation may be considered a sufficiently effective method for removal of particulate matter from the effluent. In conclusion, the deviations from the guideline are considered to be insignificant for the validity of the study.
5.2 Results and discussion	The DOC fed to the test system by means of the test substance TEGO 2000 was effectively removed from the test solution. *DR* = 81 ± 5% (mean ± SD) The test substance is known to exhibit microbicidal properties. However, this had apparently no negative effects on the test system, presumably due to the high activity and concentration of the activated sludge.
5.3 Conclusion	Since the deviations from the test guideline as discussed above had no significant impact on the outcome of the study, the test is considered to be valid. By means of the coupled units test (OECD 303A), TEGO 2000 (Ampholyt 20/100) was established to be ultimately biodegradable in sewage treatment plants.
5.4 Reliability	1
5.5 Deficiencies	No The deviations from the test guideline as discussed above may be considered as minor deficiencies that not substantially limit the reliability of the study.

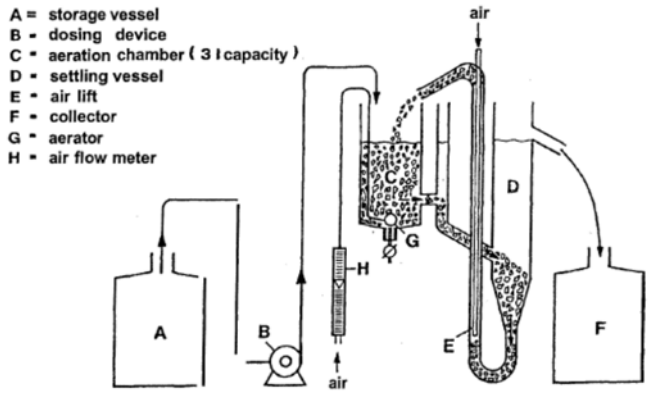
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	18/01/13	

Materials and Methods

The Applicant's version is considered acceptable with the following additions:

The activated sludge was obtained from an oxidation ditch for domestic sewage treatment. The CA notes a greater variety of microorganisms generally live in the oxidation ditch. In contrast, packaged plants usually depend upon only a few types of microorganisms to *'eat'* the sewage. Consequently, the inoculums source used in the study may lead to enhanced elimination rates that are not observed in traditional plants. According to the Applicant the test substance was raised to 120 mg/L, which is several times higher than in the Guidance Document 303A, to compensate for the higher D.O., and therefore prevent a higher degradation rate.

The sludge was sieved (2 mm) and homogenised. At the beginning of the test 1.4 L of the activated sludge (4.8 g/L suspended solids) was placed in the reaction vessel and the systems were filled up with tap water, to give a final concentration of suspended solids 2.3 g/L in the reaction vessel. The CA notes this is in accordance with the guideline which states '*When an inoculum of (about) 2.5 g/l (dry weight) activated sludge is used, the test substance may be added from the start of the test since directly adding increasing amounts from the beginning has the advantage that the activated sludge may be better able to adapt to the test substance.'*



The above figure shows the experimental set up used in this study. The test system consisted of an aeration vessel (3 L) and a separator (2 L). The test system contained mixed liquor with activated sludge and was fed with the following liquids:

- Tap water (~400 mL/hr);
- ~50 mL/4 hr of a synthetic feed solution;
- ~30 mL/hr of a solution in Milli-Q water of the test compound over a 15 min period. The dosing period was performed for 25 d.

The guideline states the DOC in the tap water and deionised water should be less than 3 and 2 mg DOC/L respectively. The CA notes the dilution water (Milli Q) contained 15-21 mg/L DOC between 09/12 and 18/12. However, this was corrected for in the calculations.

From the 6/12/91 the *test solution* had a concentration of between 1.29 and 1.39 g/L and a mean DOC of 120 mg/L (average of measured values). As a result of the variation in the volume the DOC of the test substance in the *influent* varied between 6.2 mg DOC/L and 8.6 mg DOC/L. Another unit receiving no test compound but Milli- Q water served as a blank. The units were aerated and mixed vigorously with a stream of cleaned air. The unit were operated in the dark or diffuse light at a temperature of 18±2°C. The CA notes the guideline *states ' The normal mean test concentration in the influent should be between 10 mg/l and 20 mg/l DOC, with an upper concentration of no more than 50 mg/l.'* The hydraulic

retention time was 6 hr. The CA notes the test concentrations used in this study are slightly lower than what is recommended.

Sludge settled in the separator was recycled twice per hour by an airlift to the reaction vessel. Sludge on the walls of the basin was brought back to the aqueous phase every working day and the day before every sampling day.

In order to try and equalise the microbial populations in the test unit, receiving sewage plus a test substance, and in a control unit, receiving only sewage, a daily interchange of sludge was introduced. This procedure is known as coupling. Exchange of 1.5 L (half of the volume of the aeration tank is exchanged) from the activated sludge vessel of the unit containing the test substance and 1.5 L from the activated sludge of the control unit was performed daily, except during the weekend.

Once a week 0.5 L of mixed liquor was taken from the reaction vessel and replaced by fresh activated sludge with a concentration of suspended solids of 2-2.5 g/L. The CA notes poor settlement and loss of sludge may occur in the Husmann plant units. According to the guideline this may be rectified adding fresh sludge.

A part of the mixed liquor in the reaction vessel was removed every working day. On Monday 1,125 mL was removed, on other days 375 mL. A part of the removed liquors was used for the determination of dry matter of suspended solids.
This was presumably performed to maintain a constant sludge retention time. The guideline states *'If, for example, a sludge retention time of 8 days is chosen, remove daily 1/8 of the volume of the activated sludge in the aeration vessel and discard it. Carry this out on a daily basis or, preferably, by means of an automatic intermittently operating pump.'* The CA notes the volume removed on the Monday seems large.

Effluent was collected in large glass vessels. After mixing the contents of each flask samples were taken for DOC measurement (0.1 Corporation Model 700 TOC analyser).

pH was measured on 36/6/25 of December. Oxygen content was determined every working day.

In addition to the deviations noted by the applicant the following are noted:

- Milli-Q water instead of tap water was used as a replacement of test substances in the bank unit.
- The temperature in the experiment was 18 ± 2°C instead of 20 ± 2°C.
- The units were not fed with 200 mL/4 hr of a synthetic feed solution but with 50 mL/4 hr of a 4 times concentrated solution.

Results and discussion

The applicant's versions is considered acceptable with the following additions:

The percentage elimination of dissolved organic carbon was calculated using the equations specified in the OECD 303 A guideline:

$$D_t = \frac{C_s - (E - E_o)}{C_s} \times 100$$

where D_t = % elimination of DOC at time t

C_s = DOC in the influent due to the test substance

E = measured DOC in the test effluent at time t (mg/l)

E_o = measured DOC in the control effluent at time t (mg/l)

Cs was calculated from the mean of the DOC concentrations measured in the test substance corrected for the volumes for the test substance solutions dosed every hour by the pumps and the dilution of the test solution in the total influent according to:

(Flow of the test substance/flow of effluent[1]) x mean DOC values of the test substance solutions

1. Measured during the times that the wasted sludge flow was zero

Sludge exchanges can give the appearance of quite a considerable removal, since some of the test substance in transferred and the concentrations of test substance in the test and control effluents become more nearly equal. Thus, correcting factors have to be used which depend on the fraction exchanged and the mean hydraulic retention time. In the current study the interchange fraction of the volume of the activated sludge units is (1.5 L/3.5L=) 0.5. The mean hydraulic retention time is 6 hr. This results in the following corrected DOC:

$$D_{tc} = \frac{4D_t - 100}{3}$$

D_{tc} = corrected % DOC or COD elimination

This compensates for dilution of the test substance in the aeration vessel by the sludge exchange.

The performance of the test unit was monitored by the DOC removal in the blank unit and the dry matter content of the suspended solids in both units. From the result is can be concluded that DOC removal in the blank unit after 7 d was sufficient to start determining elimination of the test substance. The DOC removal was between 94-96 %.

Figure A7.1.2.1.1- 1 shows %DOC removal as a function of time. The CA notes No lag phase is observed. This may suggest adsorption is contributing to the removal of the test substance. However, OECD guidance notes the lag phase is often highly variable and poorly reproducible, hence an immediate degradation with only a small adaption phase for the bacteria is plausible. This is supported by several publications, e.g. van Ginkel et al. (2007), who isolate a Pseudomonas sp. from activated sludge which could degrade long-chain alkylamines (C_8-C_{18}). Furthermore, Yoshimura et al. (1980) reported the ready biodegradation of primary-, secondary- and tertiary long chain alkylamines (C_4-C_{18}). In a robust summary for reliable studies summarised by US EPA, the majority of analysed alkylamines are readily biodegradable (or at least inherently biodegradable in some minor cases), which indicates an ubiquitous distribution of microorganisms with the ability to biodegrade long-chain alkylamines.

	The CA notes the aim of this test is to assess aerobic biological degradation in a sewage treatment plant. According to the '*Data requirements for biocidal product types a simulation test should at least fulfil the following criteria:* • *give measured rates for primary and ultimate degradation of the parent compound;* • *allow for identification and quantification of metabolites formed during the test.* *....The only laboratory EC STP (or the corresponding OECD STP) simulation test currently available is the 'coupled units test' (EC method C.10 or the corresponding OECD test 303A). This test cannot distinguish between biological degradation and other elimination processes such as adsorption and volatilisation. EC method C.10 or the corresponding OECD 303A test does not fulfil the criteria given above '*
Conclusion	It is concluded the test substance is rapidly removed from the test system at the test concentrations tested. The CA notes the aerobic biodegradation test (IIIA 7.1.2.1.1/01) cannot distinguish between biological degradation and other elimination processes such as adsorption and volatilisation. Consequently, the CA considers that the reported degradation may be, in part, due to removal of the test substance by adsorption to glassware, sludge etc. Consequently, the results should be treated with caution.
Reliability	2
Acceptability	The results should be treated with caution. Removal was likely, in part, due to adsorption. However, the study shows the test substance is rapidly removed from the test system.
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.1.2.1.1- 1: Description of the inoculum.

Criteria	Details
Nature	Activated sludge
Species	Mixed microbial population
Strain	Not applicable
Source	Oxidation ditch for domestic sewage treatment
Sampling site	Berkel, The Netherlands
Laboratory culture	No
Method of cultivation	None
Preparation of inoculum for exposure	Sieving (2 mm) and homogenising
Pre-treatment	Not specified
Initial cell concentration	Not stated

Table A7.1.2.1.1- 2: Description of the test system.

Criteria	Details
Culturing apparatus	OECD confirmatory test units
Number of culture flasks/concentration	1
Aeration device	Not specified
Measuring equipment	OI Model 700 TOC analyser
Test performed in closed vessels due to significant volatility of test substance	Not relevant

Table A7.1.2.1.1- 3: Description of the test conditions.

Criteria	Details	
Composition of the nutrient feed medium	Peptone	6400 mg/l
	Meat extract	4400 mg/l
	Urea	1200 mg/l
	NaCl	280 mg/l
	$CaCl_2 \cdot 2\ H_2O$	160 mg/l
	$MgSO_4 \cdot 7\ H_2O$	80 mg/l
	$K_2HPO_4 \cdot 3\ HO$	1120 mg/l
Additional substrate	None; however, the dilution water (Milli-Q) contained 15–21 mg/l DOC between Dec 09 and Dec 18, 1991; this was corrected for in the calculations	
Test temperature	18 ± 2°C	
pH	6.9–7.4 (test unit) 6.6–7.3 (blank unit)	
Aeration of dilution water	Continuous aeration of the test solutions	
Suspended solids concentration	2.0–2.5 g/l	
Other relevant criteria	Working-in time: 8 days	

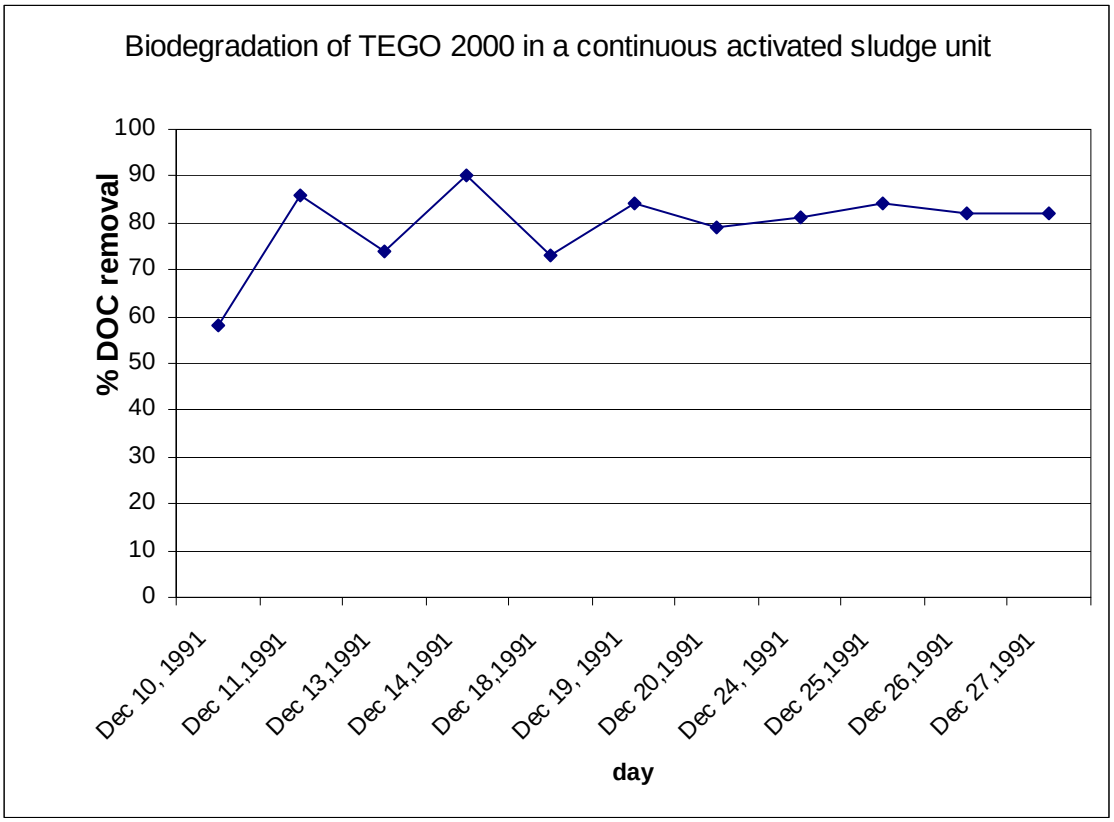


Figure A7.1.2.1.1- 2: Calculation of DOC removal of the test substance; results of the biodegradation of TEGO 2000 in a continuous activated sludge unit. The results are based on DOC determinations in the effluents and in the influents of a test unit and a blank unit and are expressed as the percentages of the test substance removed. Two outliers (according to Chauvenet) were eliminated (16^{th},17^{th} Dec.).

Section A7.1.2.1.1 Biological sewage treatment: Aerobic biodegradation

Annex Point IIIA 11.2.1 – Supportive data –

The following references are considered to contain additional information about aerobic biodegradation in sewage treatment plants and are thus presented in tabular format as supportive data:			
Reference	**Title**	**Method**	**Results**
A7.1.2.1.1/02: [redacted] (1992) Bayerische Landesanstalt für Wasserforschung, München, Germany, Report dated September 03, 1992 (unpublished).	Biologische Abbaubarkeit von TEGOL 2000/TEGO 2000 gemäss modifiziertem OECD-Bestätigungstest.	Simulation test (modified OECD confirmatory test) of tenside removal, which is basically equivalent to OECD 303A; Test substance: TEGO 2000 (containing 20% Ampholyt 20/100 in aqueous solution; Inoculum: activated sludge from a municipal STP; Test substance concentrations:1 mg/l, 2 mg/l, 5 mg/l, 10 mg/l; Test duration: 2 weeks employing 1 mg/l and 2 mg/l in parallel assays, respectively; further 2 weeks at 5 mg/l and 10 mg/l, respectively; Working-in time: 2 weeks Method of analysis: Photometry after reaction with “Orange 11”, DOC analysis, CSB determination. GLP: No The study is poorly documented and thus of limited validity.	DOC-removal: 92–95% CSB-removal: 93–96% a.i. removal: > 99%

EVALUATION BY COMPETENT AUTHORITIES

Date

21/01/13

Materials and Methods

Applicant's version is deemed acceptable with the following additions:

The four inflow concentrations of the surfactant were 5-50 mg/L. This corresponds to 1-10 mg/L (i.e. 20 %) of the active substance.

The test substance is continuously dosed with mineral nutrient solution in venting chambers where biological degradation takes place. The sludge obtained from a communal purifying plant flows into a precipitation chamber and from there it is pumped back into the venting chamber. The inflow and outflow is checked for surfactant content.

The analysis methods were the DOC/CSB measurement as well as the photometry of the coloured salt adduct of ampho surfactant/ orange II for determining primary degradation.

CSB is the German abbreviation for COD (chemical oxygen demand).

Results and discussion

The Applicant's version is considered acceptable with the following additions:

Total *carbon* determination (average of 3 readings each)

	Inflow	**outflow**	**% removal**
	mg/L		
Control	56	2.5	96
5 mg/L Tegol 2000/Tego 2000	56	2.6	95
10 mg/L Tegol 2000/Tego 2000	59	3.0	95
15 mg/L Tegol 2000/Tego 2000	57	3.4	94
50 mg/L Tegol 2000/Tego 2000	59	4.5	92

COD determination (average of 3 readings each)

	Inflow	**outflow**	**% removal**
	mg/L		
Control	130	4	97
5 mg/L Tegol 2000/Tego 2000	133	5	96
10 mg/L Tegol 2000/Tego 2000	135	5	96
15 mg/L Tegol 2000/Tego 2000	142	8	94
50 mg/L Tegol 2000/Tego 2000	152	11	93

Nitrogen compounds

Ammonium content in all outflows was < 0.1 mg/L.
Nitrite was not detectable in 90 % of outflows.
>90% of total nitrogen of the inflow exists in the outflow as nitrate.

Surfactant specific degradation

The surfactant content in the inflow and outflow is determined using Orange II.
The surfactant degradation was >>99% for all the test concentrations.

Conclusion

The study is poorly documented and thus of limited validity and cannot be used directly in risk assessment. However the study provides supportive information (cf 7.1.2.1.1) as it shows over 90 % of the test substance is removed from the effluent. Some inhibition is observed at higher test substance concentrations.

Reliability

3

Acceptability

Unacceptable for direct use in risk assessment. However, the study provides some useful supportive information.

Remarks

Section A7.1.2.1.1 Biological sewage treatment: Aerobic biodegradation

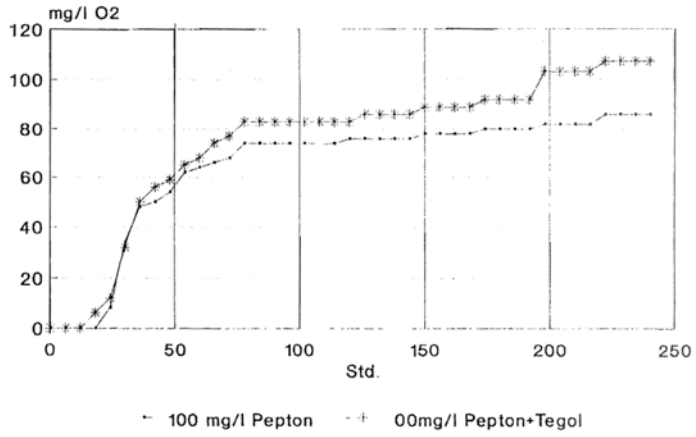
Annex Point IIIA 11.2.1 – Supportive data –

Reference	Title	Method	Results
A7.1.2.1.1/03: **cross reference to A7.4.1.4/03:** [redacted] (1992): Abwasser- und Peptonabbau-hemmungsuntersuchungen im Sapromat und modifizierter OECD-Bestätigungstest mit TEGOL 2000: Bayerische Landesanstalt für Wasserforschung, München, 1992 (unpublished)	„Waste Water and Peptone Degradation Inhibition Tests in the Sapromat and modified OECD Confirmatory Test with TEGOL 2000“	Simulation test (modified OECD confirmatiory test) of tenside removal, which is basically equivalent to OECD 303A; Test substance: TEGO 2000 (containing 20% Ampholyt 20/100 in aqueous solution; Inoculum: activated sludge from a municipal STP; Test substance concentrations:1 mg/l, 2 mg/l, 5 mg/l, 10 mg/l; Test duration: 2 weeks employing 1 mg/l and 2 mg/l in parallel assays, respectively; further 2 weeks at 5 mg/l and 10 mg/l, respectively; Working-in time: 2 weeks Method of analysis: Photometry after reaction with “Orange 11”, DOC analysis, CSB determination. GLP: No The study is poorly documented and thus of limited validity.	DOC-removal: 92–95% CSB-removal: 93–96% a.i. removal: > 99%

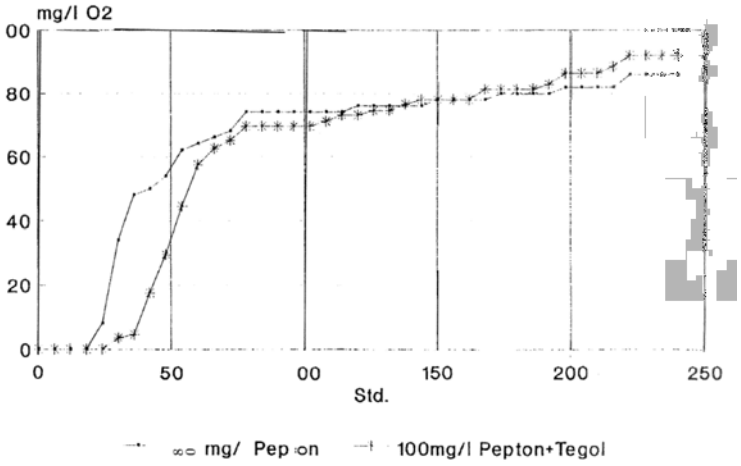
	EVALUATION BY COMPETENT AUTHORITIES
Date	21/01/13
Materials and Methods	Applicant's version is deemed acceptable

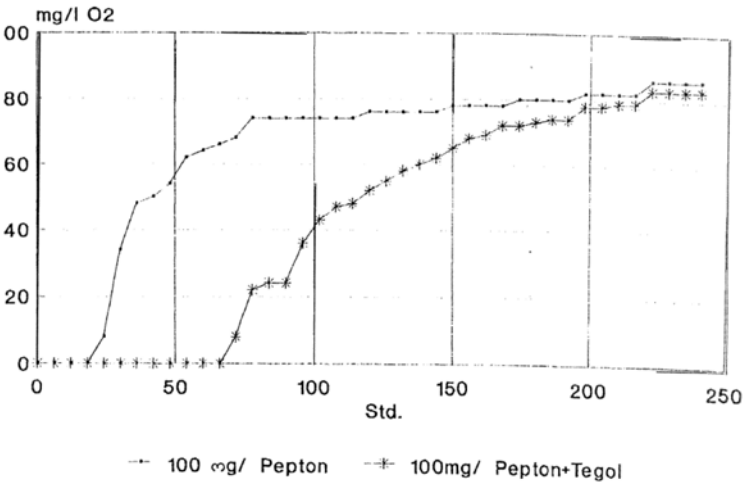
Results and discussion

The Applicant's version is considered acceptable with the following additions:



mit 5 mg/l TEGOL 2000





At 5 and 100 mg peptone + Tegol /L some inhibition was observed. This is marked by the longer lag phase relative to the control in the oxygen attrition curves. After 200 hr of exposure to the high concentration the curves becomes similar. This suggests metabolism of the peptone substrate is possible via adaptation processes.

Conclusion	The study is poorly documented and thus of limited validity and cannot be used directly in risk assessment. However the study provides supportive information (cf 7.1.2.1.1) as it shows over 90 % of the test substance is removed from the effluent. Inhibition was observed at 5 mg peptone +Tegol/L. This effect was significantly stronger at 100 mg peptone +Tegol/L.
Reliability	3
Acceptability	Unacceptable for direct use in risk assessment. However, the study provides some useful supportive information.
Remarks	

Section A7.1.2.1.2 Anaerobic biodegradation
Annex Point IIIA 12.2.1

Official use only

1. Reference

1.1 Reference	**A7.1.2.1.2/01:** [redacted] (2007): Anaerobic biodegradability test, ultimate anaerobic biodegrability of Ampholyt 20 by digested sludge. Fraunhofer-Institute for Molecular Biology and Applied Ecology (IME), Schmallenberg, Germany, Report No. EBR-013/3-30, October 09, 2007 (unpublished).
1.2 Data protection	Yes
1.2.1 Data owner	Goldschmidt GmbH
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. for the purpose of its entry into Annex I.

2. Guidelines and Quality Assurance

2.1 Guideline study	OECD 311 (2006)
2.2. GLP	Yes
2.3. Deviations	No

3 Materials and Methods

3.1 Test material	As given in Section A2.
3.1.1 Lot/Batch number	ES67345616
3.1.2 Specification	As given in Section A2. The active substance as manufactured is obtained as a “product-by-process”, constituting a 20 % (w/w) aqueous solution of the active matter.
3.1.3 Purity	20 % (w/w) aqueous solution of pure active matter (99 % w/w)
3.1.4 Further relevant properties	The a.i. is a multi-component substance as specified in Section A2. The test substance is hydrolytically stable (water solubility ≥ 200 g/L) and the vapour pressure is 1.9×10^{-4} Pa (20°C). (see section A3)
3.1.5 Composition of Product	20 % a.i. (aqueous solution, “product by process”)
3.1.6 TS inhibitory to microorganisms	Yes, as given in Section A7.4.1.4 the EC_{50} is 22 mg/l.
3.1.7 Specific chemical analysis	Not required according to the guideline OECD 311.
3.2 Reference substance	Sodium benzoate
3.2.1 Initial concentration of reference substance	100 mg C_{org}/L
3.3 Testing procedure	
3.3.1 Inoculum/ test species	Digested sludge, see Table A7.1.2.1.2- 1.

Section A7.1.2.1.2 Anaerobic biodegradation

Annex Point IIIA 12.2.1

3.3.2. Test system	The test system is described in Table A7.1.2.1.2- 2.
3.3.3. Test conditions	See Table A7.1.2.1.2- 3.
3.3.4. Method of preparation of test solution	Ampholyt 20 stock solution (3.314 mL per 100 mL, corresponding to a concentration of 4003 mg C_{org}/L (analytically verified by DOC measurement using a SHIMADZU TOC-Analyser 5050A)) was mixed with mineral medium/inoculum suspension and filled up to 218 mL, resulting in a concentration of 100 mg C_{org} per litre.
3.3.5. Initial TS concentration	100 mg C_{org}/L
3.3.6 Duration of test	60 days
3.3.7 Analytical parameter	Headspace pressure, continuously measured. Dissolved inorganic carbon (DIC), measured at the end of the test.
3.3.8 Sampling	Daily (pressure) and at test termination (DIC).
3.3.9 Intermediates/ degradation products	Not identified
3.3.10 Controls	Blank control Reference substance (sodium benzoate 100 mg C_{org}/L) Toxicity control: 100 mg C_{org}/L Ampholyt 20 and 100 mg C_{org}/L sodium benzoate
3.311 Statistics	Per cent biodegradation, according to guidelines.

4. Results

4.1 Degradation of test substance

4.1.1 Degradation of TS in abiotic control	Not stated
4.1.2 Degradation	No degradation of Ampholyt 20 was detected after 60 days. Data are given in Table A7.1.2.1.2- 4.
4.1.3 Graph	The net gas pressure progression is graphically presented in Figure A7.1.2.1.2- 1.
4.1.4 Other observations	The results give rise to the probable cause that Ampholyt 20 is toxic to the bacterial population used in the test, at least at concentrations demanded by the test guideline.
4.1.5 Degradation of reference substance	94 %
4.1.6 Intermediates/ degradation products	Not identified

5 Applicant's Summary and conclusion

Section A7.1.2.1.2 Anaerobic biodegradation

Annex Point IIIA 12.2.1

5.1 Materials and methods	The biodegradation of Ampholyt 20 at a concentration of 100 mg C_{org}/L was investigated according to OECD guideline 311 over a 60-day period in anaerobic aqueous medium. As inoculum microorganisms from a digester of a sewage treatment plant mainly fed with municipal wastewater were used. The rate of degradation was monitored by measuring the increase in headspace pressure in the vessels resulting from the production of carbon dioxide and methane is measured. The amount of inorganic and methane carbon resulting from the biodegradation of the test item was calculated from the net gas production and net IC formation in the liquid phase in excess over blank control values. The extent of biodegradation was calculated from total IC and methane-C produced as a percentage of the calculated amount of carbon added as test compound.
5.2 Results and discussion	The anaerobic biodegradation of Ampholyt 20 was found to be 0 % after 60 days. Therefore, Ampholyt 20 must be considered as not readily biodegradable under the chosen test conditions. The degradation in the toxicity control (–38 to –34 %) was lower than the degradation in the functional control (94 %). Due to the same concentration of reference item in both experimental approaches, degradation under 50 % in the toxicity control indicates an inhibiting effect of the test item on the degradation of the reference item. This signified an antibacterial effect of Ampholyt 20 under test conditions.
5.3 Conclusion	Due to the results the test item can be identified as non-biodegradable under anaerobic conditions and potentially toxic to bacteria at the concentration required by the guideline.
5.2.1 Reliability	2
5.3.2. Deficiencies	Yes The test item was employed at a concentration that is potentially toxic to micro-organisms. The test item concentration is, however, within the range demanded by the test guideline. Thus, the study is considered to be valid with restrictions only.

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	21/01/14	
Materials and Methods	Applicant's version is considered acceptable with the following additions: It is unclear from the study report how the solutions were deraerated as this was not mentioned. All validity criteria in this study were met: • Pressure readings were only taken from vessels that do not show pink coloration • The test is considered valid as the reference substance reached a plateau that represents more than 60% biodegradation. • The pH at the end of the test has exceeded the range 7 ± 1	
Results and discussion	Applicant's version is considered acceptable	
Conclusion	Applicant's version is considered acceptable	
Reliability	2	
Acceptability	Study is deemed acceptable, noting the comments in Section 5.3.2	
Remarks		
	COMMENTS FROM ...	
Date		
Materials and Methods		
Results and discussion		
Conclusion		
Reliability		
Acceptability		
Remarks		

Table A7.1.2.1.2- 1: Inoculum/ test organism.

Criteria	Details
Nature	Digested sludge
Species	Mixed microbial population
Strain	Not applicable
Source	Sewage treatment plant
Sampling site	Municipal STP at Lennestadt, Germany
Laboratory culture	No
Method of cultivation	Not applicable
Preparation of inoculum for exposure	The sludge was pre-digested, without the addition of any nutrients, at 35 ± 2 °C for 5 days.
Pre-treatment	Pre-digestion as above; suspension in oxygen-free mineral medium; centrifugation, followed by re-suspension in mineral medium; final concentration of total solids of 2.9 g dry mass/litre
Initial cell concentration	Not stated

Table A7.1.2.1.2- 2: Description of the test system.

Criteria	Details
Culturing apparatus	Not reported
Number of culture flasks/concentration	Inoculum blank: 3 vessels Procedural control: 3 vessels Test suspension: 3 vessels Toxicity control: 3 vessels
Measuring equipment	gas pressure: Sensomat measurement device by Aqualytic® IC: TOC analyzer
Oxidation reduction indicator	Resazurin

Table A7.1.2.1.2- 3: Description of the test conditions.

Criteria	Details
Composition of the medium	Mineral test medium, according to the guideline
Additional substrate	No
Solvent	No
Preparation of medium	As indicated in the guideline
Test temperature	35 °C
pH	7.0 ± 0.2 at the beginning of the test
Suspended solids concentration	2.9 g dry mass/litre
Other relevant criteria	The test was run in darkness. The suspension was kept anaerobic during the whole test.

Table A7.1.2.1.2- 4: Percent net degradation, given as mean values ± SD [% C].

	Ampholyt 20	Sodium benzoate	Toxicity control
Degradation [% C]	–72* ± 5	94 ± 9	–36 ± 2

*) Negative values indicate less degradation than in inoculum blank

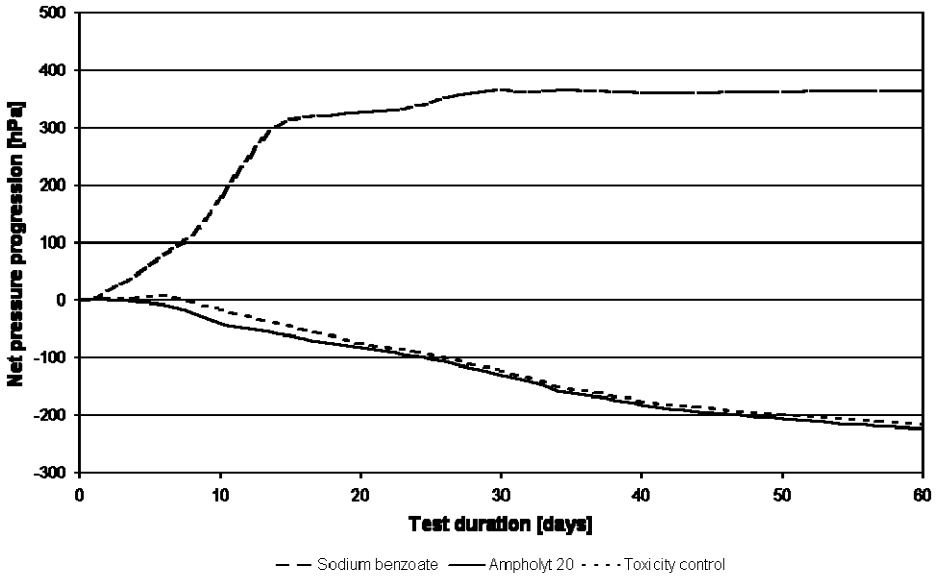


Figure A7.1.2.1.2- 1: Net gas pressure progression; negative values indicate less gas production than in the inoculum blank.

Section A7.1.2.2.1 Aerobic aquatic degradation study

Annex Point IIIA 12.2.1

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	The ready biodegradability of Ampholyt 20 has been demonstrated in Section A7.1.1.2.1. Therefore, no further degradation studies are required since the established "ready biodegradability" provides sufficient information for risk assessment. Further, based on the decision tree in subchapter 7.0.2 (Testing Strategy on biodegradation of biocidal active substances), the assessment of rate constants is already possible in the case that the criterion for ready biodegradability is fulfilled, and further testing is not explicitly recommended.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	22/01/12	
Evaluation of applicant's justification	Ampholyt 20/Ampholyt 20/100 was rapidly removed from the test systems used to investigate ready biodegradability (Studies 7.1.1.2.1/01 &7.1.1.2.1/02). The CA considers that the reported degradation may be, in part, due to removal of the test substance by adsorption to glassware, sludge etc. Consequently, the results should be treated with caution. In view of the rapid *removal* of the test substance from these systems a study on aerobic aquatic degradation is not considered required.	
Conclusion		
Remarks		
	COMMENTS FROM ...	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A7.1.2.2.2 Water/sediment degradation study

Annex Point IIIA 12.2.1

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	The ready biodegradability of Ampholyt 20 has been demonstrated in Section A7.1.1.2.1. Therefore, no further degradation studies are required since the established "ready biodegradability" provides sufficient information for risk assessment. Further, based on the decision tree in subchapter 7.0.2 (Testing Strategy on biodegradation of biocidal active substances), the assignment of rate constants is already possible in the case that the criterion for ready biodegradability is fulfilled, and further testing is not explicitly recommended. Furthermore, regarding the envisaged field of use (surface disinfectant to be applied indoors only), direct release to surface waters is not expected.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	22/01/12	
Evaluation of applicant's justification	Ampholyt 20/Ampholyt 20/100 was rapidly removed from the test systems used to investigate ready biodegradability (Studies 7.1.1.2.1/01 &7.1.1.2.1/02). The CA considers that the reported degradation may be, in part, due to removal of the test substance by adsorption to glassware, sludge etc. Consequently, the results should be treated with caution. In view of the rapid *removal* of the test substance from these systems a study on aerobic aquatic degradation is not considered required. At WG III (2014) it was agreed to classify Ampholy 20 as readily biodegradble failing the 10 window.	
Conclusion		
Remarks		
	COMMENTS FROM ...	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A7.1.3 **Adsorption/desorption screening test**
Annex Point IIA 7.7

Official use only

Reference

Reference	**Cross-reference to A3.5/01:** [redacted] (2002) Determination of physico-chemical properties of Tego 2000. Infracor GmbH, Marl, Germany, Report No. AN-ASB 0198, April 16, 2002 (unpublished).
Data protection	Yes
Data owner	Goldschmidt GmbH
Companies with letter of access	No
Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

Guidelines and Quality Assurance

Guideline study	Yes OECD 121
GLP	Yes
Deviations	Yes/No

Materials and Methods

Test material	As given in Section A2. "TEGO 2000" is a trade name of the active substance Ampholyt 20, obtained as a "product by process", i.e., a 20% aqueous solution of the pure active.
Lot/Batch number	17EM17
Specification	As given in Section A2 for the 20% aqueous solution ("product by process").
Purity	20% of the pure active in water
Further relevant properties	The active substance, obtained as a "product by process", consists of several chemical species which may not be expected to be uniform regarding their adsorptive properties.
Method of Analysis	HPLC method according to OECD guideline 121.
Degradation products	No
Method of analysis of degradation products	Not applicable.

Section A7.1.3 Adsorption/desorption screening test

Annex Point IIA 7.7

Reference substance	Seven reference substances were used in HPLC analysis to determine the calibration curve (see Table A7.1.3- 1).	
Method of analysis of reference substance	Testing method implies use of reference substances for determination of the calibration curve of the HPLC system; see 3.1.5.	
Soil types	Not applicable.	
Testing procedure		
Test system	HPLC pump, Spectra-Physics Inc. Detector: differential refractometer, Knauer Column: Zorbax CN, 5 µm particle size, 250 × 4 mm	
Test solution and test conditions	Mobile phase:	Methanol / water (purified) 55/45 (v/v), pH adjusted at 3.0 with 85% phosphoric acid
	Determination of t_0:	Urea (23.9 mg/ 25 ml eluent) was used as reference substance
	Injection volume:	20 µl
	Detection:	Refractive index
	Flow rate:	1.0 ml/min
	Temperature:	24–25 °C
	Replication:	Two runs for each substance
Test performance		
Preliminary test	According to ”OECD 106”: No	
Screening test: Adsorption	According to ”OECD 106”: No	
Screening test: Desorption	According to ”OECD 106”: Not performed	
HPLC-method	According to ”OECD 121”: Yes For details see above.	
Other tests	No	

Results

Preliminary test	Not performed.
Screening test: Adsorption/ desorption (HPLC)	
Dead time	t_0 = 2.33 min
Retention data of reference substances	Retention times are given in Table A7.1.3- 1. The calibration curve (log *k'* vs. log k_{oc}) indicated satisfactory linearity and precision (r = 0.989).
Retention time of the test substance	Due to the composition of the test substance of several chemical species, the retention time is given as a range (see Table A7.1.3- 2).
Calculations	

Section A7.1.3 Adsorption/desorption screening test

Annex Point IIA 7.7

Capacity factor	See Table A7.1.3- 2.
Adsorption coefficient	See Table A7.1.3- 2.
Degradation products	No degradation products were tested.

Applicant's Summary and conclusion

Materials and methods	The adsorption coefficient (K_{oc}) of Ampholyt 20 on soil and sewage sludge was estimated by the HPLC method according to OECD guideline 121. The pH of the mobile phase was adjusted to a value of 3, with H_3PO_4 (85 %).
Results and discussion	Ampholyt 20 is highly soluble in water (see Section A3.5), is hydrolytically stable (see Section A7.1.1.1.1), and is non-volatile (see Section 3.2). The substance-specific properties are therefore not considered to have any significant impact on the results. Due to the composition of the test substance of several chemical species, the adsorption coefficient is given in a range of log K_{oc} = 2.70–3.99, corresponding to K_{oc} = 501–9772
Conclusion	The study was not performed in full compliance to OECD guideline 121. The pH value was adjusted to 3.0 which is outside of the range of 5.5 and 7.5. This range is provisioned by the guideline, since the pH has a significant influence on sorption behaviour in particular for polar substances. Additional testing at a pH value within this range was not performed. Therefore, the test considered to be of limited validity.
Reliability	3
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	25/01/13
Materials and Methods	The Applicant's version is considered acceptable
Results and discussion	The Applicant's version is considered acceptable with the following addition: The HPLC method at best provides an estimate of the Koc and cannot fully replace the batch equilibrium method. To correlate the measured HPLC-retention data of a test substance with its adsorption coefficient, Koc, a calibration graph has to be established. A minimum of six reference points, at least one above and one below the expected value of the test substance should be used. The accuracy of the method will be significantly improved if reference substances that are structurally related to the test substance are used. In the case of Ampholyt 20, four of the six reference substances (phenol, triapenthenol, fenthion, trifluralin, used do not show any structural similarity with the test substance. In addition none of the reference substances have the carboxylic acid function group. Consequently, the accuracy of the Koc for Ampholyt 20 is called into question. In addition the effect of ionisation was not fully investigated. As noted by the applicant in **Section 5.3,** pH has a significant influence on sorption behaviour in particular for polar substances. pH is likely to influence adsorption behaviour of Ampholyt 20. For agricultural soils or tanks of sewage treatment plants pH normally varies between pH 5.5 and 7.5. For ionisable substances, two tests should be performed with both ionised and non-ionised forms in appropriate buffer solutions but only in cases where at least 10 % of the test compound will be dissociated within pH 5.5 to 7.5. This was not performed in the case of Ampholyt 20. The HPLC measurements were performed with a mobile phase with a pH 3. At pH 3 , some of the alkyl amines may be protonated. This may increase the retention time. In light of the above the study is considered of limited use.
Conclusion	In the case of Ampholyt 20, four of the six reference substances (phenol, triapenthenol, fenthion, trifluralin) used, to construct the calibration curve do not show any structural similarity with the test substance. In addition only one of the reference substances possesses the carboxylic acid function group. Consequently, the accuracy of the Koc for Ampholyt 20 is called into question. The effect of pH on adsorption was not investigated. The results may also have been affected by the surfactant properties of the test substance. Therefore, the study is considered to be of limited validity and is not suitable for use in risk assessment.
Reliability	3
Acceptability	The study is considered to be of limited validity and is not suitable for use in risk assessment.
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.1.3- 1: List of reference substances used in the HPLC analysis.

Name of substance	t_R [min]	log k_{oc}
Phenol	3.01	1.32
Acetanilide	3.05	1.61
Monuron	3.76	1.99
Triapenthenol	5.14	2.37
Linuron	5.37	2.62
Fenthion	8.52	3.30
Trifluralin	13.44	3.92

Table A7.1.3- 2: Range of retention times and the resulting adsorption coefficients of Ampholyt 20.

	t_R [min]	k'	log k'	log k_{oc}
Lower limit	5.47	1.35	0.130	2.70
Upper limit	16.33	6.01	0.779	3.99

Section A7.1.4.1 Field study on accumulation in the sediment

Annex Point IIIA 12.2.1

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	In chapter 3 of the TNsG on additional data requirements (point A7.1.4.1), the conduct of a field study on accumulation in the sediment is required, if non-extractable residues are formed exceeding 70% of the initial dose in the water/sediment study, or if the mineralisation rate in the water/sediment system is less than 5% in 100 days. However, the submission of such studies is not considered to be required, for the following reasons: The ready biodegradability of the active ingredient of Ampholyt 20 has been demonstrated in Section A7.1.1.2.1. Thus, accumulation of any residues in the sediment is therefore not expected.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	22/01/13	
Evaluation of applicant's justification	Applicant's justification is considered acceptable According to the *Data requirements for biocidal product types (FINAL DRAFT) Version 4.3.1 April 2000 if* 'non-extractable residues are formed exceeding 70% of the initial dose in the water/sediment study or if the mineralization rate in the water/sediment system is less than 5% in 100 days, then a field study on accumulation in the sediment should be done.'	
Conclusion	However, since Ampholyt 20/ Ampholy 20/100 was rapidly removed in ready biodegradability tests a water /sediment study is not considered required. A field study on accumulation in sediment is not required.	
Remarks		
	COMMENTS FROM ...	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A7.2.1 Aerobic degradation in soil, initial study

Annex Point IIIA 7.4, 7.1.1

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	The conduct of aerobic degradation studies in soil is not considered to be required, for the following reasons: (i) The intended biocidal use is not involved with a quantitatively relevant direct release to the soil compartment. (ii) The active ingredient of Ampholyt 20 is readily biodegradable (Section A7.1.1.2.1) and has a variable adsorption tendency to soil, depending of the individual chemical species considered. Although a reliably measured K_{oc} is not yet available (see sections A7.1.3 and A7.2.3.1), on average a moderate adsorption tendency may be concluded, based on the log P_{ow} estimated using QSAR (see A3.9/02). According to the average log P_{ow} of 3.81, a Kp_{soil} of 20.1 is derived following the TGD. (iii) Based on the ready biodegradability and the derived soil adsorption coefficient, the approach suggested by the TGD (chapter 2.3.6.5, Table 8) allows a default soil half-life to be assigned. Thus, considering the results of the ready biodegradability test and the soil adsorption characteristics (soil partition coefficient: Kp_{soil} = 20.1), an average soil half-life of 30 days may be allocated by default. It is therefore not considered to be required to conduct soil degradation studies.	
Undertaking of intended data submission [ ]		

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	22/01/13
Evaluation of applicant's justification	The applicant's justification was written in 2007. A new batch equilibrium adsorption study was submitted in 2009. Consequently, the Applicant's statements in relation to the Koc etc are out of date. The CA notes according to the *'Data requirements for biocidal product types': 'If the biocide is directly applied/emitted to soil, then a soil simulation test is required'.* The Table below summarises the proposed uses of Ampholyt 20:

PT	Field of use envisaged
PT 2	Disinfectant for surfaces in private, public and industrial areas
PT 3	Disinfectant for surfaces in veterinary area (areas in which animals are housed or transported, footbaths for animals for prevention of cross contamination
PT 4	Disinfectant for surfaces, containers, or pipelines associated with the production, transport, storage of food, feed or drink for humans or animals

These product types do not result in direct soil exposure. Indirect soil exposure may occur through the application of sewage sludge/manure. Application of sewage sludge or manure on agricultural land is considered indirect exposure as the manure and sludge act as intermediate compartments.

Ampholyt 20/Ampholyt 20/100 was rapidly removed from the test systems used to investigate ready biodegradability (Studies 7.1.1.2.1/01 &7.1.1.2.1/02).

Conclusion	A soil degradation study is not required.
Remarks	

	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A7.2.2.1 Rate and route of degradation in at least three soil types

Annex Points IIIA7.4, IIIA7.1.1, IIIA7.1.4

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	The investigation of aerobic soil degradation in three soil types (A7.2.2.1) is neither a core data requirement, nor an additional data requirement for product types 2, 3 and 4 according to the TNsG (chapter 2.5). In addition, the conduct of aerobic degradation studies in soil is not considered to be required, for the following reasons: (i) The intended biocidal use is not involved with a quantitatively relevant direct release to the soil compartment. (ii) The a.i. of Ampholyt 20 is readily biodegradable (Section A7.1.1.2.1). (iii) Based on the ready biodegradability and the soil adsorption coefficient, the approach suggested by the TGD (chapter 2.3.6.5, Table 8) allows a default soil half-life to be assigned. Thus, considering the results of the ready biodegradability test and the soil adsorption characteristics (soil partition coefficient: Kp_{soil} = 20.3, please refer to Document IIA, Chapter 4.1.1.3), a soil half-live of 30 days may be allocated by default. A study on the adsorption and desorption of Ampholyt 20 in five soil types is currently ongoing and will be submitted upon availability (A7.2.3.1). It is therefore not considered to be required to conduct further soil degradation studies.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	24/01/13
Evaluation of applicant's justification	This justification was evaluated under Annex Point IIIA 7.4. Aerobic degradation in soil, initial study.
Conclusion	Further soil degradation studies are not required as direct soil exposure does not occur.
Remarks	
	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A7.2.2.2 Field soil dissipation and accumulation

Annex Point IIIA 7.1.1

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	The investigation of field soil dissipation and accumulation (7.2.2.2) is neither a core data requirement, nor an additional data requirement for product types 2, 3 and 4 according to the TNsG (chapter 2.5). In addition, the conduct of such a study is not considered to be required for the following reasons: (i) The intended biocidal use is not involved with a quantitatively relevant direct release to the soil compartment. (ii) The a.i. of Ampholyt 20 is readily biodegradable (Section A7.1.1.2.1) and its adsorption tendency to soil (mobility in soil) which was estimated based on physicochemical properties following the TGD, part III, chapter 4 (please refer to Document IIA, Chapter 4.1.1.3), being Kp_{soil} = 20.3 l/kg. (A study on the adsorption and desorption of Ampholyt 20 in five soil types is currently ongoing and will be submitted upon availability (A7.2.3.1). (iii) Based on the ready biodegradability and the soil adsorption coefficient, the approach suggested by the TGD (chapter 2.3.6.5, Table 8) allows a default soil half-life to be assigned. Thus, considering the results of the ready biodegradability test and the soil adsorption characteristics (soil partition coefficient: Kp_{soil} = 20.1), a soil half-live of 30 days may be allocated by default. It is therefore not considered to be required to conduct further soil degradation, or dissipation and accumulation studies.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	24/01/13
Evaluation of applicant's justification	This justification was evaluated under Annex Point IIIA 7.4. Aerobic degradation in soil, initial study.
Conclusion	A field soil dissipation/accumulation study is not required.
Remarks	

	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A7.2.2.3 Extent and nature of bound residues

Annex Point IIIA 7.1.4

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	The investigation of the extent and nature of bound residues (7.2.2.3) is neither a core data requirement, nor an additional data requirement for product types 2, 3 and 4 according to the TNsG (chapter 2.5). In addition, the conduct of such a study is not considered to be required for the following reasons: (i) The intended biocidal use is not considered to be involved with a quantitatively relevant direct release to the soil compartment. (ii) The a.i. of Ampholyt 20 is readily biodegradable (Section A7.1.1.2.1) and has a variable adsorption tendency to soil, depending of the individual chemical species considered (Sections A7.1.3 and A7.2.3.1, presently ongoing study). (iii) Based on the ready biodegradability and the soil adsorption coefficient, the approach suggested by the TGD (chapter 2.3.6.5, Table 8) allows a default soil half-life to be assigned. Thus, considering the results of the ready biodegradability test and the soil adsorption characteristics (soil partition coefficient: Kp_{soil} = 20.1, please refer to Document IIA, Chapter 4.1.1.3), a soil half-live of 30 days, depending on the individual compound of the active substance, may be allocated by default. It is therefore not considered to be required to conduct further soil degradation studies.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	24/01/13
Evaluation of applicant's justification	This justification was evaluated under Annex Point IIIA 7.4. Aerobic degradation in soil, initial study.
Conclusion	A study investigating the nature and extent of bound residues is not required.
Remarks	
	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A7.2.2.4 Other soil degradation studies

Annex Point IIIA7.1.1

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	The investigation of other degradation pathways in soil (7.2.2.4) is neither a core data requirement, nor an additional data requirement for product types 2, 3 and 4 according to the TNsG (chapter 2.5). In addition, the conduct of such a study is not considered to be required for the following reasons: (i) The intended biocidal use is not considered to be involved with a quantitatively relevant direct release to the soil compartment. (ii) The a.i. of Ampholyt 20 is readily biodegradable (Section A7.1.1.2.1) and has a variable adsorption tendency to soil, depending of the individual chemical species considered (Sections A7.1.3 and A7.2.3.1, presently ongoing study). (iii) Based on the ready biodegradability and the soil adsorption coefficient, the approach suggested by the TGD (chapter 2.3.6.5, Table 8) allows a default soil half-life to be assigned. Thus, considering the results of the ready biodegradability test and the soil adsorption characteristics (soil partition coefficient: Kp_{soil} = 20.1, please refer to Document IIA, Chapter 4.1.1.3), a soil half-live of 30 days, depending on the individual compound of the active substance, may be allocated by default. It is therefore not considered to be required to conduct further soil degradation studies.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	24/01/13
Evaluation of applicant's justification	This justification was evaluated under Annex Point IIIA 7.4. Aerobic degradation in soil, initial study.
Conclusion	No soil degradation studies are required.
Remarks	
	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A7.2.3.1 **Adsorption and desorption**
Annex Point IIIA XII.1.2

Official use only

Reference

Reference	**A7.2.3.1/01:** [redacted] (2008) Determination of the Adsorption/Desorption of ^{14}C Ampholyt 20/100. Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Schmallenberg, Germany, Report No. EBR-013/7-13, April 03, 2008 (unpublished).
Data protection	Yes
Data owner	Goldschmidt GmbH
Companies with letter of access	No
Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

Guidelines and Quality Assurance

Guideline study	Yes EU method C.18 (2001/59/EC) and OECD 106
GLP	Yes
Deviations	No

Materials and Methods

Test material	^{14}C-labelled Ampholyt 20/100 (Dodecyl-1-^{14}C-labelled)
Lot/Batch number	XVI/38
Specification	Specific physico-chemical properties of the ^{14}C-1-dodecyl Ampholyt 20 are not available, but may conveniently be considered to be equivalent to those of the unlabelled substance since it represents the dodecyl fraction which dominates in the unlabelled Ampholyt 20 by approx. 75 %. Therefore please refer to Ampholyt 20 as given in Section A2.
Purity	Not defined (mixture of Amines, dodecyltrimethylenedi-, reaction products with chloro-acetic acid); however, the signals attributable to the dodecyl-components of Ampholyt 20 account for 94.86 % of the radioactivity of the test item
Further relevant properties	Total activity: 209.0 MBq/mg Specific activity: 1.423 MBq/mg

Section A7.2.3.1 Adsorption and desorption

Annex Point IIIA XII.1.2

Method of analysis	Liquid scintillation counting (LSC, Packard Tri-Carb liquid scintillation analyser). The limit of detection of LSC is 0.4 Bq. Due to the sample volume and considering the specific activity of 1.423 MBq/mg the limit of detection for the test is 0.028 µg/L Ampholyt for a sample volume of 10 ml and 0.28 µg/L for a sample volume of 1 ml.	
Degradation products	The average adsorption/desorption behaviour of Ampholyt 20/100 (a.s.) was determined by measuring the total radioactivity without differentiation of the single components of the test substance.	
Method of analysis of degradation products	Not applicable	
Reference substance	No	
Method of analysis of reference substance	Not applicable	
Soil types	Five different test soils were used according to the test guideline. The soils differed in clay content (soil texture), organic carbon content and pH value. The soils with the internal codes IME-06-A, IME-04-A, IME-03-G and IME-01-A were collected by the test facility and are commonly used for environmental fate studies in this laboratory. The soil "LUFA No. 2.2" was obtained from "Lufa Speyer". For details on the test soils, please refer toTable A7.2.3.1- 1.	X1
Testing procedure		
Test system – Adsorption	Known volumes of solutions of the test item at known concentrations in 0.01 M $CaCl_2$ are added to soil samples of known dry weight which have been pre-equilibrated in 0.01 M $CaCl_2$ (24 h). The mixture is agitated for an appropriate time. The soil suspensions are then separated by centrifugation and the aqueous phase is analysed. The amount of test item adsorbed to soil is calculated as the difference between the amount of test item initially present in solution and the amount remaining in solution at the end of the experiment (indirect method).	
Test solution and test conditions – adsorption	The necessary amount of soil was weighed into centrifuge tubes (80 mL). Then 45 mL of 0.01 M $CaCl_2$ solution was added to the soil. The samples were shaken horizontally for 24 h to establish a soil-water equilibrium. Then 5 mL of the test item solution (= stock solution in 0.01 M $CaCl_2$-solution was added and the tubes agitated horizontally on a mechanical shaker. The samples were centrifuged and the ^{14}C-radioactivity in the aqueous supernatant was determined by liquid scintillation counting (LSC).	X2
Test system/ test solution and test conditions – desorption	The volume of solution removed after the adsorption experiment was replaced with an equal volume of 0.01 M $CaCl_2$ without test item. The new mixture was agitated again, then separated by centrifugation and the ^{14}C-radioactivity concentration in the aqueous supernatant was determined (LSC).	

Section A7.2.3.1 Adsorption and desorption

Annex Point IIIA XII.1.2

Test performance		
Preliminary test	A preliminary test was performed to find the optimum soil/solution ratio (test: 2 soils and soil/solution ratios 1:50, 1:25, and 1:10), to select the test item concentration, to check the adsorption onto the vessel surface, to establish the mass balance of the ^{14}C-radioactivity.	X3
Screening test: Adsorption	In the adsorption experiments control samples (only test item in 0.01 M $CaCl_2$ solution, no soil) were prepared and at each sampling time two control samples were analysed in order to check the adsorption of the test item on the surfaces of the test vessels. Blank samples (0.01 M $CaCl_2$ solution and soil, no test item) with every used soil were run. All experiments including controls and blanks were performed in duplicate.	
Screening test: Desorption	Desorption experiments were done after the adsorption process was carried out. In the determination of desorption isotherms only one agitation time (time needed to reach desorption equilibrium) was used.	
HPLC-method	Not applicable, no degradation products were analysed.	
Other tests	No	

Results

Preliminary test	Optimum soil/solution ratio: 1:50 Test item concentration: 108.7 µg/l In the soil/solution experiments the total recovery of the applied ^{14}C-radioactivity ranged from 80.6 % to 92.7 %. The control samples showed that approximately 20 % of the applied ^{14}C-radioactivity were adsorbed onto the glass walls, which corresponds to the losses of ^{14}C-radioactivity determined in the experiments (data are given in Table A7.2.3.1- 2).	
Screening test: Adsorption	In most cases the adsorption equilibrium was nearly reached already after 1 h of agitation. Please refer to data given in Table A7.2.3.1- 3. Linear regression analysis was performed for every soil (empirical Freundlich isotherm).	X4
Screening test: Desorption	In the desorption solution the test item concentrations diverged considerably after 1–8 h desorption time, but in general the desorption equilibrium was reached after 8 h. Please refer to data given in Table A7.2.3.1- 4. The logarithms of the test item concentrations in the soils were plotted versus the logarithms of the desorbed test item concentration in the supernatant. Linear regression analysis was performed for every soil (empirical Freundlich isotherm).	
Calculations	Calculated adsorption coefficients (K_F^{ads}) were in the range of 853.3–2428.8 The normalised KOC values were in the range of 31660–86743. Please refer to Table A7.2.3.1- 5 for detailed data. Calculated desorption coefficients were in a range of 1350.2–3183.5. The normalised KOC values were in the range of 35532–138413. Please refer to Table A7.2.3.1- 6 for detailed data.	
Degradation products	Not applicable	

Section A7.2.3.1 Adsorption and desorption

Annex Point IIIA XII.1.2

Applicant's Summary and conclusion

Materials and methods	The adsorption/desorption process of Ampholyt 20/100 was investigated according to OECD-guideline 106. The study was performed using ^{14}C-labelled representative lead compounds (dodecyl) of Ampholyt 20/100. The average adsorption/desorption behaviour of Ampholyt 20/100 was determined by measuring the total ^{14}C-radioactivity without differentiation of the single components of the test substance. For the investigation five soils with different content of organic carbon and clay were chosen.	
Results and discussion	The adsorption of Ampholyt 20/100 onto each of the five soils was a rapid process: The results of the adsorption kinetics revealed that in most cases the adsorption equilibrium was already reached after 1 h. Percentage of adsorbed ^{14}C-radioactivity (mean values) are in the range of 85.8 % and 96.0 %. Calculated adsorption coefficients (K_F^{ads}) were in the range of 853.3–2428.8 The desorption kinetics experiments showed that the adsorption was only marginally reversible. Only 4.7–7.7 % of the adsorbed Ampholyt 20/100 were desorbed. Calculated desorption coefficients (K_F^{des}) were in a range of 1350.2–3183.5. The results clearly suggest that differentiation between the single components of Ampholyt 20 (five ^{14}C-labelled dodecyl lead compounds employed in this study) is not necessary. Instead, the active substance adsorbs strongly onto soil and organic matter, and the obtained results derivation of a generic adsorption or desorption coefficient which may be used in the risk assessment. Ampholyt 20 is known to be hydrolytically stable. Thus, degradation during the experiment need not be expected and identification of potential degradation products was hence not considered to be necessary.	
Conclusion	No circumstances were reported that may have affected the integrity and quality of the results, thus this study is considered to be valid without restrictions. According to SSLRC mobility classification, Ampholyt 20 can be classified as non-mobile (K_{OC} > 4000 ml/g).	
Reliability	1	X5
Deficiencies	None	

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	11-01-13	
Materials and Methods	The notifiers version is considered acceptable with following comments: X1 Soil characterisation is not contained in the study report. Poor characterisation was given in the Applicants summary. The Applicant was requested by the CA to submit proper characterisation of the test soils. This was received in March 2013. X2 All soils were air dried (20-25°C), mixed and passed through a 2 mm sieve. Concentrations of 20.3-213.1 mg/L were selected for the test item solution, which was applied to the soils in the adsorption/isotherms. The lowest selected concentration is about two orders of magnitude higher than the detection limit. The highest concentration is less than half the solubility. The Reviewer notes these test concentrations are not spanning two orders of magnitude as specified by the guideline. LOD The LOD for LSC was 0.4 BQ. Due to the sample volume and considering the specific activity of 1.423 MBq/mg the LOD for the test is 0.028 µg/mL Ampholyt for a sample volume of 10 mL and 0.28 µg/L for a sample volume of 1 mL. X3 The optimal soil/solution ratio was investigated with two soils; *'3 Osnabrück'* and *'5 Marisfield'*. The former had high organic carbon content and low clay content. The latter had high clay content and low organic matter content. The following soil:solution ratios were investigated: 1:50, 1:25 and 1:10. X4 The CA disagrees with the applicants statement. Equilibrium appeared to take longer than 1 hour in the majority of soils (please refer to **Figure CA 7.2.3.1-1** for further details). However, equilibrium has been established by 24 hr.	

Evaluation by Competent Authorities

Results and discussion

The notifiers results are considered acceptable with the following additions:

The average K_d values at the end of the 24 hr adsorption period in the adsorption kinetic experiments were

	Marisfeld	Osnabrück	Ebbinghof	Borstel	Lufa No. 2.2
K_d (cm^3/g)	891.46	100.16	649.39	546.81	645.94
K_d oc	35658.6	35862.8	17089.3	54681.2	28084.2

soil/water ratio = 1:50. Concentration 108.7 µg/L

The K_d is considered accurate as the K_d x(soil/water ratio) is greater than 0.3

In the adsorption isotherms, 1/n varied from 1.0986 to 1.1745. Four of the five soils have 1/n values greater than 1.1. The CA notes this is somewhat unusual as 1/n are typically in the range 0.7 to 1.1 in adsorption experiments. However, valid 1/n values can occur outside this (guideline) range.

The distribution coefficients as a function of concentration are given in the table below. These were determined in the adsorption isotherm experiments

Conc. (µg/L)	K_d oc (cm^3/g)				
	Marisfeld	Osnabrück	Ebbinghof	Borstel	Lufa 2.2
20.3	23294.5	24129.4	15431.8	34053	21963.6
	26463.4	25914	15725	38882.7	21781.8
51.8	33139.6	37030.9	19015.7	33400.9	32857.2
	33386.3	35104.5	19482.6	51808.5	27247.9
106.5	32262.1	31922.6	18927.5	43259.3	28687.6
	30470.9	33661.7	18631.5	41441.6	26516.5
108.7*	34475.1	35888.8	16983.9	53997.2	27936.7
	36842.1	35836.8	17194.7	55365.2	28321.6
159.3	36695.4	37113.3	19569.4	50810.8	29472.5
	33693.9	34925.4	20035.4	47530.6	31181.2
213.1	34159.3	38697.1	20072.6	47789.3	30092.6
	27969.3	40083.5	19884.4	49441.9	30743.1
Average	**31,904.3**	**34,192.3**	**18,412.9**	**45,648.4**	**28,066.9**
Average (n=5)	**31,645.0**				
Average (n = 50)	**31,645.0**				

*24 hr value in adsorption kinetics experiment

Adsorption for the mixture does not appear to be correlated with pH. The CA notes Ampholyt 20 consists of a mixture of alkyl amino acetic acids and alkyl amines. Individual components of the mixture may exhibit pH dependent adsorption. For example the acetic acid components may be more mobile under alkaline conditions. None of the soils tested had a pH greater than 7. Under acidic conditions the alkyl amines may adsorb more strongly. In addition corresponding hydrochlorides of the alkyl amino acetic acids and alkyl amines may be present in the Ampholyt 20 mixture. These substances may be more strongly adsorbed to clay soils.

Evaluation by Competent Authorities	
Conclusion	$K_{F\ OC}$ values for the ^{14}C-1-dodecyl components of Ampholyt 20/100 were in the range of 31,660–86,743 cm^3/g. The dodecyl fraction dominates the unlabelled Ampholyt 20 by approximately 75 % w/w. The desorption kinetics experiments showed that the adsorption was only marginally reversible. Only 4.7–7.7 % of the adsorbed Ampholyt 20/100 were desorbed.
Reliability	2
Acceptability	The CA notes the adsorption behaviour of Ampholyt 20/100 was determined by measuring the total ^{14}C-radioactivity without differentiation of the single components of the test substance. Ampholyt 20 is comprised of approximately 20 constituents. Some of the components have difunctional amine structures with caboxylation grades ranging from 0 – 2. The ionic or even amphoteric nature of the Ampholyt 20 constituents implies that the charge of each individual molecule will be strongly pH dependent. For example, in the environment cationic substances are expected to sorb to organic carbon and be less bioavailable/mobile. Hence the endpoints from the adsorption studies should be treated with caution The CA notes the Kocs are only reflective of the major (C_{12}-based, 70-75 %) portion of Ampholyt 20/100, and may require adjustment for other components (i.e., C_{14}-based).

Remarks

Post ECHA WG III meeting (Environmental session) 2014

Adsorption endpoints for the Environmental Exposure assessment

Due to the surface-active properties, long-chained alkyl amines adsorb strongly onto the solid phase of soil and sediments. The substances can adsorb both onto the organic fraction and, dependent on the chemical composition, onto the surface of the mineral phase, where sodium and potassium ions can be exchanged against the alkyl ammonium ion. The determination of a Koc from log Kow is not appropriate, because the equations for Koc derivation are not valid for both ionic and surface active substances. Following discussions at the ECHA WG III meeting 2014 it was agreed to perform the environmental exposure assessment with the lowest measured Koc (15,431.8 cm^3/g) to cover the lower range of Kocs and the highest permitted Koc in EUSES (1 x 10^6 cm^3/kg) to cover the higher range of Kocs exhibited by some components of Ampholyt 20. The following distribution behaviour is predicted by EUES 2.1.2:

Predicted distribution behaviour of Ampholyt 20 residues within a STP (TGD)

Properties	**Ampholyt 20/100**	
Biodegradation	Readily biodegradable, failing the 10 d window	
Koc (L/kg)	15,431.8	1 x 10^6
Percentage of emission directed to air by STP*	8.81 x 10^{-8}	3.64 x 10^9
Percentage of emission directed to water by STP*	17.1	8.31
Percentage of emission directed to sludge by STP*	53	90.3
Percentage of emission degraded in the STP*	29.9	1.37

For benzylalkylammonium chlorides (BACs) surfactants, Clara *et al*[3] reported *biotransformation* rates of 80–94% in wastewater treatment plants (C_{12}-C_{18}). However the components of Ampholyt 20 have generally a more complex structure and may be expected to be more persistent relative to BACs which are regarded as readily biodegradable. For dialkyldimethyl (DDAC) and alkyltrimethyl (ATAC) ammonium chlorides with varying alkyl chain lengths a different situation was identified. Removal rates of more than 90% were obtained for all homologues. However approximately 70% was due to adsorption to the sludge and removal via the excess sludge. Removal due to biotransformation amounts to approximately 20% for the DDACs. Only for DDAC-C10 higher removal due to biotransformation (>90%) was observed. According to the study author these results are comparable to

Evaluation by Competent Authorities	
	results reported in the literature for DDAC-C_{18}. In this case 53 % was removed via adsorption by the excess sludge removal and 36–43% was removed via biodegradation. These measurements although not directly applicable to Ampholyt 20 suggest degradation of the test substance may be underestimated by the model calculation (EUSES). However, this is conservative form a risk assessment point of view. It should also be noted that the results reported by Clara *et al*[3] are based on single measurements and therefore have to be interpreted with care, as storage processes were neglected. Furthermore, for the sludge measurements activated sludge from other than the investigated WWTPs was used. Therefore the results of the evaluation provides a rough assessment of the removal pathway of BACs and DDACs. The CA notes the overall removal rates for Ampholyt 20 from the (TGD) STP is predicted to be 82.9 %-91.67 %.[4] This is consistent with the results observed in the STP simulations tests where removal rates of 81 ± 5 % (IIIA 7.1.2.1.1/01) and 92–99 % (III A7.1.2.1.1/02) were reported. This suggests the amount directed to water ranged from ~1 / 8 % to 14/19 %. This is in good agreement with the values predicted by EUSES for Ampholyt 20 (8.31-17.1 %). To obtain more realistic removal rates etc. it may be useful to monitor the influent and effluent of wastewater treatment plants for Ampholyt 20 components.
Date **Materials and Methods** **Results and discussion** **Conclusion** **Reliability** **Acceptability** **Remarks**	COMMENTS FROM ...

[3] M. Clara, S. Scharf, C. Scheffknecht, O. Gans, *'Occurrence of selected surfactants in untreated and treated sewage'*, Water Research 41 (2007) 4339 – 4348

[4] The STP simulation tests did not identify the mechanism of removal (adsorption/biodegradation)

Table A7.2.3.1- 1: Classification and physicochemical properties of soils used as adsorbents; n.r. = not reported, these figures may be recovered from the raw data if required.

	Marisfeld	**Osnabrück**	**Ebbinghof**	**Borstel**	**Lufa No. 2.2**
Soil order	n r.	n r.	n r.	n r.	n.r.
Soil series	n r.	n r.	n r.	n r.	n.r.
Classification	Clay loam	Loamy sand	Silt loam	Sandy loam	Loamy sand
Location	n r.	n r.	n r.	n r.	n.r.
Horizon	n r.	n r.	n r.	n r.	n.r.
Sand [%]	n r.	n r.	n r.	n r.	n.r.
Silt [%]	n r.	n r.	n r.	n r.	n r.
Clay [%]	35.0	5.0	29.0	5.0	7.9
Organic carbon [%]	2.5	2.8	3.8	1.0	2.3
Carbonate as $CaCO_3$	n r.	n r.	n r.	n r.	n r.
Insoluble carbonates [%]	n r.	n r.	n r.	n r.	n.r.
pH (1:1 H2O)	6.8	5.7	5.6	5.5	5.7
Cation exchange capacity (MEQ/100 g)	n r.	n r.	n r.	n r.	n.r.
Extractable cations (MEQ/100 g)	n r.	n r.	n r.	n r.	n.r.
Ca	n r.	n r.	n r.	n r.	n r.
Mg	n r.	n r.	n r.	n r.	n r.
Na	n r.	n r.	n r.	n r.	n r.
K	n r.	n r.	n r.	n r.	n r.
H	n r.	n r.	n r.	n r.	n r.
Special chemical/mineralogical features	n r.	n r.	n r.	n r.	n r.
Clay fraction mineralogy	n r.	n r.	n r.	n r.	n.r.

Table A7.2.3.1- 2: Results of preliminary test.

Test substance	As given in chapter 0 above
Sample purity	As given in chapter 3.1 above
Weighed soil	1, 2, and 5 g
Volume of $CaCl_2$ solution	5 mL
Nominal concentration of a.s. final solution	1.056 or 1.245 µg/mL
Analytical concentration final of a.s. solution	Not reported
Concentration of the test solution (show calculation)	Not reported
Details of the analytical method used:	LSC
Method	LSC
Recovery rate	Not reported
Detection limit	0.4 Bq, corresponding to0.028 µg a.s./L (10 mL sample volume)

Table A7.2.3.1- 3: Results of screening test – adsorption; n.r. = not reported, these figures may be recovered from the raw data if required.

	Marisfeld		**Osnabrück**		**Ebbinghof**		**Borstel**		**Lufa 2.2**	
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
Concentration of test material [µg/L]										
After contact of 24 hours with soil										
Correction for blank with soil	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Correction for blank without soil	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Final corrected concentration [µg/L]	6.1	5.7	5.1	5.1	8.0	7.9	9.3	9.1	8.0	7.9
Initial concentration of test solution [µg/L]	108.7	108.7	105.6	105.6	108.7	108.7	108.8	108.8	108.7	108.7
Decrease in concentration [µg/L]	**!Syntaxfehler, .**	**!Syntaxfehler, .**	100.5	100.5	100.7	100.8	**!Syntaxfehler, .**	**!Syntaxfehler, .**	**!Syntaxfehler, .**	**!Syntaxfehler, .**
Quantity adsorbed [µg]	5.13	5.15	5.03	5.03	5.04	5.04	4.97	4.98	5.04	5.04
Quantity of soil [g of oven-dried equivalent]	0.973	0.973	0.983	0.983	0.971	0.971	0.989	0.989	0.983	0.983
Quantity adsorbed [µg] per gram of soil	5.28	5.29	5.11	5.11	5.19	5.19	5.03	5.04	5.13	5.13
Test material adsorbed [%]	94.4	94.7	95.2	95.2	92.6	92.7	91.4	91.6	92.7	92.7
Temperature [°C]	20	20	20	20	20	20	20	20	20	20

Volume of solution recovered after centrifugation [mL]	n r.	n.r.	n.r.	n.r.	n.r.	n r.	n r.	n.r.	n r.	n r.
Volume of solution not recovered [mL]	n r.	n.r.	n.r.	n.r.	n.r.	n r.	n r.	n.r.	n r.	n r.
Corresponding quantity of test substance [mg]	n r.	n.r.	n.r.	n.r.	n.r.	n r.	n r.	n.r.	n r.	n r.

Table A7.2.3.1- 4: Results of screening test – desorption.

	Marisfeld		Osnabrück		Ebbinghof		Borstel		Lufa 2.2	
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
Temperature [°C]	20	20	20	20	20	20	20	20	20	20
Conc. in combined washings after 24 h [µg/L]	4.4	7.4	4.8	5.2	6.7	6.4	6.5	6.2	8.6	7.7
Corresponding quantity of test material [µg]	0.22	0.37	0.24	0.26	0.34	0.32	0.33	0.31	0.43	0.38
Quantity desorbed [µg]	0.20	0.36	0.22	0.25	0.30	0.30	0.31	0.29	0.41	0.36
[%] of adsorbed test material desorbed	4.0	6.9	4.4	5.0	6.0	5.9	6.2	5.8	8.2	7.2
[%] of adsorbed test material not desorbed	96.0	93.1	95.6	95.0	94.0	94.1	93.8	94.2	91.8	92.8

Table A7.2.3.1- 5: Adsorption data of Ampholyt 20/100 obtained by linear regression analysis.

Soil	2 Marisfeld	3 Osnabrück	4 Ebbinghof	5 Borstel	Lufa No. 2.2
Internal No.	06-A	04-A	03-G	01-A	-
R^2	0.9885	0.9881	0.9971	0.9983	0.9924
Intercept: log K_F^{ads}	3.1128	3.3854	3.0803	2.9311	3.0986
1/n	1.0986	1.1745	1.1026	1.1434	1.1324
Ads. coeff. K_F^{ads}	1296.6	2428.80	1203.1	853.3	1254.9
C_{org} [%]	2.5	2.8	3.8	1.0	2.3
K_{oc}	51864	86743	31660	85330	54561

CA comment
In the study report it was stated the data was fitted to

$$\text{Log } (C_s^{ads}(eq)/1000) = \log K_F^{ads} + 1/n \log(C_{aq}^{ads}(eq)/1000)$$

However, the 1/1000 conversion factor was only applied to the Caq data in the isotherms. This was to convert from units of µg/L to mg/L. Strictly speaking K_F has units of $\mu g^{1-1/n}(cm^3)^{1/n} g^{-1}$

Table A7.2.3.1- 6: Desorption data of Ampholyt 20/100 obtained by linear regression analysis.

Soil	**2 Marisfeld**	**3 Osnabrück**	**4 Ebbinghof**	**5 Borstel**	**Lufa No. 2.2**
Internal No.	06-A	04-A	03-G	01-A	-
R^2	0.9716	0.9977	0.9831	0.9886	0.9861
Intercept: log K_F^{des}	3.3244	3.4239	3.1304	3.1312	3.5029
1/n	1.1087	1.1491	1.0636	1.0858	1.2375
Des. coeff. K_F^{des}	2110.6	2654.0	1350.2	1352.7	3183.5
C_{org} [%]	2.5	2.8	3.8	1.0	2.3
K_{OC}	84424	94786	35532	135270	138413

Evaluation by Competent Authority

Figure CA 7.2.3.1-1. Percentage adsorption of ^{14}C Ampholyt 20/100 to soils as a function of time

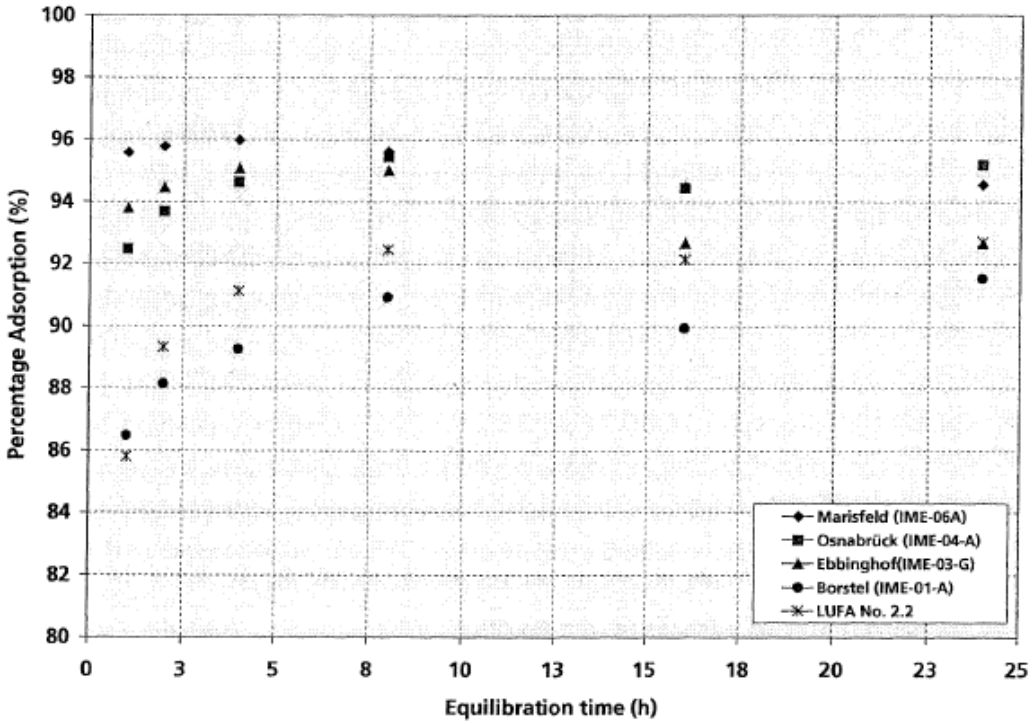


Table 7.2.3.1-1. Mass balance observed in soil

Table 4: Mass balance of the total ^{14}C-radioactivity in soil/solution systems and in control samples after adsorption/desorption process; applied ^{14}C-radioactivity: 15.2 KBq

sample	total ^{14}C-radioactivity % ITR [initial applied radioactivity]			
	$CaCl_2$ ads. solution	$CaCl_2$ des. solution	Soil	total
control	82.2 81.0	4.5 4.1		86.7 85.1
5 Borstel	9.4 9.1	4.9 4.8	70.9 76.0	85.2 89.9
4 Ebbinghof	6.2 6.0	4.5 4.5	71.1 74.7	81.8 85.2
3 Osnabrück	4.2 4.2	3.2 3.3	81.6 77.6	89.1 85.1
2 Marisfeld	5.4 6.6	3.7 3.4	80.3 80.5	89.4 90.5
Lufa no. 2.2	6.6 6.3	4.3 3.9	74.4 70.4	85.3 80.6

able 5: Mass balance of the total ^{14}C-radioactivity in soil/solution systems and in control samples after adsorption/desorption process; applied ^{14}C-radioactivity: 3.7 KBq

sample	total ^{14}C-radioactivity % ITR [initial applied radioactivity]			
	$CaCl_2$ ads. solution	$CaCl_2$ des. solution	Soil	Total
control	71.5 78.0	8.1 5.6		79.6 83.6
5 Borstel	12.4 8.5	4.3 4.5	76.0 77.1	92.7 90.1
4 Ebbinghof	6.4 6.2	4.0 4.8	73.7 71.1	84.1 82.1
3 Osnabrück	4.6 4.6	3.7 4.6	77.2 75.0	85.5 84.3
2 Marisfeld	5.5 5.6	3.4 3.2	80.1 73.8	89.0 82.6
Lufa no. 2.2	6.1 7.2	4.5 4.7	76.6 72.6	87.2 84.5

Comment from the CA

In the soil/solution experiments the total recovery of the applied radioactivity ranged from 80.6 % to 92.7 %. However, the control experiments (test item + 0.01 M $CaCl_2$) show that up to 20% of the applied radioactivity was adsorbed onto the glass walls (Ampholyt 20 is stable to hydrolysis), which corresponds to the losses of radioactivity determined in the adsorption desorption experiment.

Soil characterisation

Name	Internal code	pH ($CaCl_2$)	OC%	Sand %	Silt %	Clay %*	soil type	CEC [mmol c/kg]
Marisfeld	IME-06-A	6.8	2.5	8	56	36	Clay loam	245
Osnabrück	IME-04-A	5.7	2.8	81	15	4	Loamy sand	89
Ebbinghof	IME-03-G	5.6	3.8	17	54	29	Silt loam	126
Borstel	IME-01-A	5.5	1.0	67	27	6	Sandy loam	37
Lufa 2.2	--	5.7	2.3	78	15	7	Loamy sand	101

*Minor changes compared to the original report occur due to rounding of texture data

For the determination of the Adsorption/Desorption properties of the test item 5 different test soils were used. The characterization of the IME-refesols (IME-01-A to IME-06-A) was performed according to GLP under GLP-code IME-005/7-85. Data from the Lufa 2.2 soil were taken from the Lufa Speyer homepage (www.lufa-speyer.de).

Source: First amendement to Adsorption/Desorption of Ampholyt 20/100 (EBR-013/7-13)

Section A7.2.3.2 Annex Point IIIA7.1.3	Mobility in at least three soil types and where relevant mobility of metabolites and degradation products	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	In view of the ready biodegradability of the substance (Section A7.1.1.2.1) and its molecular structure, no degradation products of concern are likely to be formed. According to the TNsG (Chapter 3) further screening tests on the adsorption/desorption of metabolites and other degradation products are therefore not considered to be required.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	24/01/13
Evaluation of applicant's justification	Ampholyt 20 adsorption was satisfactorily investigated in the batch equilibrium adsorption experiment in five different soil.
Conclusion	Ampholyt 20 is regarded as readily biodegradable. According to the' *Data requirements for biocidal product types (FINAL DRAFT) Version 4.3.1 April 2000: Substances which are either readily biodegradable or inherently biodegradable can be considered to have such a high mineralization rate that formation of relevant metabolites is highly unlikely.*" Consequently, screening tests on the adsorption/desorption of metabolites and other degradation products are therefore not required. Studies on the mobility of the parent and relevant metabolites in soil are not required.
Remarks	
	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A7.3.1
Annex Point IIIA 7.5

Phototransformation in air (estimation method), including identification of breakdown products

Official use only

Reference

Reference	**A7.3.1/01:** [redacted] (2007) Estimation of the photochemical oxidative degradation rate in the atmosphere of Ampholyt 20. Report No. GOL-070713-03, EBRC Consulting GmbH, Hannover, Germany, July, 13 2007 (unpublished).
Data protection	Yes
Data owner	Goldschmidt GmbH
Companies with letter of access	No
Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

Guidelines and Quality Assurance

Guideline study	Not applicable (model calculation)
GLP	No Not applicable (model calculation)
Deviations	Not applicable (model calculation)

Materials and Methods

Test material	Not applicable (model calculation)
Degradation products	The formation of degradation products was not considered in this study.
Estimation method	
Considered reactions	Reaction in the atmosphere of photochemically produced OH radicals (•OH) with organic chemicals, and ozone (O_3) with olefinic/acetylenic compounds. In the case of the individual components of Ampholyt 20, the following reactions were considered: – hydrogen abstraction – addition of hydroxyl radicals to nitrogen atoms Reaction with ozone was not considered due to absence of olefinic/ acetylenic bonds.
Assumptions	Atmospheric concentrations of •OH and ozone were assumed as follows: $c_{OH} = 1.5 \times 10^6$ molecules/cm^3; 12-h day for reaction with •OH. c_{Ozone} not relevant for the reasons given under 0 oben.

X1

Section A7.3.1
Annex Point IIIA 7.5

Phototransformation in air (estimation method), including identification of breakdown products

Calculations	Estimation of the rate constants k_{OH} and k_{Ozone}, based on structure-activity relationships (SAR). Calculations performed with program AOPWIN, version 1.91 (available from the U.S. EPA website). Atmospheric half-lives of Ampholyt 20: $t_{½}$ (•OH) = ln 2/(k_{OH} × c_{OH})	

Results

Rate constants	k_{OH} = 128.7 × 10^{-12} cm^3/molecule × s (for the slowest reacting individual compound, which is the N-(3-aminopropyl)-N-decylamine) k_{Ozone} = not applicable due to absence of double bonds in all molecules	
Half life	$t_{½}$ (•OH) = 0.998 h (for the slowest reacting individual compound, which is the N-(3-aminopropyl)-N-decylamine; all other compounds were estimated to exhibit significantly shorter half-lives).	
Specification of breakdown products	The formation of breakdown products was not examined.	

Applicant's Summary and conclusion

Materials and methods	The atmospheric photo-oxidative degradation of Ampholyt 20 by hydroxyl radicals and ozone was estimated using structure-activity relationships (SAR), with the help of the software model AOPWIN. No guidelines for this purpose are available, but the method applied rests on generally accepted scientific principles, as also recommended by the TNG on data requirements.	
Results and discussion	The results suggest that Ampholyt 20 is rapidly degraded in the atmosphere by photo-oxidative processes. The maximum numerical half-life is summarised below. The TNG on data requirements recommend an assessment of potential breakdown products, as well as an assessment of further interactions of substances with atmospheric processes. Due to the extremely low vapour pressure of Ampholyt 20 (see Section A3.2), the potential for global warming, stratospheric ozone depletion, tropospheric ozone formation, and acidification, is considered to be negligible. Furthermore, according to the considered reactions, the formation of volatile compounds that might interact with atmospheric processes is not expected. Thus, the results from the current study are considered to be sufficient for the assessment of the fate of the substance in air.	X1
Half life	$t_{½}$ (•OH) = 0.998 h	
Conclusion	Phototransformation of Ampholyt 20 has been estimated according to generally accepted principles. Thus, this calculation is considered to be valid.	

Section A7.3.1 Phototransformation in air (estimation method), including identification of breakdown products

Annex Point IIIA 7.5

Reliability	0 (model calculation)
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	20/12/12
Materials and Methods	X1
Results and discussion	The applicant has performed the modelling calculations using an OH = 1.5×10^6 molecules/cm^3;12-h day for reaction with •OH. This is not in accordance with the TGD which specifies a 24 hr time period and •OH of 5 x 10^5 molec/cm^3. Consequently , the CA has repeated the calculations using the input parameters specified in the TGD. The calculations were only repeated for $C_{10}H_{21}NH(CH_2)_3NH_2$ as this molecule gave the highest half life in the notifiers calculations, **Table CA 7.3-1**. A half life of 0.125 d (2.993 hr) was obtained by the CA for the reaction with hydroxyl radicals According to the TGD, the impact of a substance on global warming depends on its IR absorption characteristics and its atmospheric lifetime. A potential greenhouse gas shows absorption bands in the so-called atmospheric window (800–1,200 nm). Any Ampholyt 20 reaching the atmosphere will undergo photolysis with hydroxyl radicals (DT_{50} 0.125 day – AOPWIN). The quoted photolytic degradation rate is sufficiently high so that Ampholyt 20 will not persist long enough to contribute to global warming, irrespective of its spectral properties.
Conclusion	A half life of 0.125 d was obtained for reaction of $C_{10}H_{21}NH(CH_2)_3NH_2$ with hydroxyl radicals. This molecule gave the longest half life of all Ampholyt 20 components. The results suggest that Ampholyt 20 is rapidly degraded in the atmosphere by photo-oxidative processes.
Reliability	3
Acceptability	CA calculations replaces notifiers modelling
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Evaluation by the Competent Authority

Table CA 7.3-1. Notifiers summary of rate constans for oxidative degradtion of individual compounds of Ampholyt 20 by hydroxyl radicals.

Compound / functional group R =	Rate constant [10^{-12} cm^3 × molecule × s^{-1}]			Half-life [h]
	Hydrogen abstraction	Reaction with N, S and -OH	Total	
[redacted]	44.67	84.00	128.7	0.998
	47.50	84.00	131.5	0.976
	50.33	84.00	134.3	0.956
	53.15	84.00	137.2	0.936
	57.70	130.0	187.7	0.684
	60.53	130.0	190.6	0.674
	63.35	130.0	193.4	0.664
	66.18	130.0	196.2	0.654
	51.19	126.5	177.7	0.722
	54.01	126.5	180.5	0.711
	56.84	126.5	183.4	0.700
	59.67	126.5	186.2	0.689

Compound / functional group R =	Rate constant [10^{-12} cm^3 × molecule × s^{-1}]			Half-life [h]
	Hydrogen abstraction	Reaction with N, S and -OH	Total	
[redacted]	51.19	87.52	138.7	0.952
	54.01	87.52	141.5	0.907
	56.84	87.52	144.4	0.889
	59.67	87.52	147.2	0.872
	57.70	130.0	187.7	0.684
	60.53	130.0	190.6	0.674
	63.35	130.0	193.4	0.664
	66.18	130.0	196.2	0.654

Figure CA- 7.3-1. Structural fromua of Ampholyt 20 components

Structural formula:

CAS-No.: 139734-65-9

Mol. wt. (mean): 280.79 g/mol

Table CA7.3-2. Modelling perfomed by the CA for $C_{10}H_{21}NH(CH_2)_3NH_2$ using AOPwin V1.91 (24 hr time period and •OH of 5 x 10^5 molec/cm^3)

```
SMILES : CCCCCCCCCCNCCCN
MOL FOR: C13 H30 N2
MOL WT : 214.40
------------------- SUMMARY (AOP v1.92): HYDROXYL RADICALS (25 deg C) --------
Hydrogen Abstraction       =  44.6726 E-12 cm3/molecule-sec
Reaction with N, S and -OH =  84.0000 E-12 cm3/molecule-sec
Addition to Triple Bonds   =   0.0000 E-12 cm3/molecule-sec
Addition to Olefinic Bonds =   0.0000 E-12 cm3/molecule-sec
Addition to Aromatic Rings =   0.0000 E-12 cm3/molecule-sec
Addition to Fused Rings    =   0.0000 E-12 cm3/molecule-sec

   OVERALL OH Rate Constant = 128.6726 E-12 cm3/molecule-sec
   HALF-LIFE =     0.125 Days (24-hr day; 0.5E6 OH/cm3)
   HALF-LIFE =     2.993 Hrs
------------------- SUMMARY (AOP v1.91): OZONE REACTION (25 deg C) -----------

              ******  NO OZONE REACTION ESTIMATION ******
              (ONLY Olefins and Acetylenes are Estimated)

Experimental Database:  NO Structure Matches
```

Section A7.3.2 Fate and behaviour in air, further studies

Annex Point IIIA 12.3

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	Exposure of the atmosphere to the active matter or Ampholyt 20 (formally named Ampholyt 20/100) is considered to be extremely unlikely: The substance is non-volatile and will not be applied as a fumigant. The standard application of the biocidal product is low-pressure spraying resulting in a large droplet size, so that partitioning into the air is insignificant. Thus, exposure of the atmosphere is limited and the substance is considered to cause no risks to the atmospheric environment. In view of the limited exposure, the data requirements on fate and behaviour in air are considered to be completely covered by Section A7.3.1.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	25/01/13
Evaluation of applicant's justification	The applicant's justification is considered acceptable. Any Ampholyt 20 reaching the atmosphere will undergo photolysis with hydroxyl radicals (DT_{50} 0.125 day – AOPWIN, cf 7.3.1). Consequently, further studies investigating the fate and behaviour are not needed.
Conclusion	
Remarks	
	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A7.4.1.1 **Acute toxicity to fish**
Annex Point IIA 7.1

Official use only

Reference

Reference	**A7.4.1.1/01:** [redacted] (2002) Ampholyt 20/100 – determination of the acute toxicity for the fish *Cyprinus carpio*. Infracor GmbH, Marl, Germany, report no. FK 1444, October 01, 2002 (unpublished).
Data protection	Yes
Data owner	Goldschmidt GmbH
Companies with letter of access	No
Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

Guidelines and Quality Assurance

Guideline study	Yes EC C.1 (92/69/EEC) OECD 203 (1992)
GLP	Yes
Deviations	No

Materials and Methods

Test material	Ampholyt 20/100 as given in Section A2.
Lot/Batch number	ES62403356
Specification	As given in Section A2. The active substance as manufactured is obtained by the production process as a “product-by-process”, constituting a 20% (w/w) aqueous solution of the active matter. For the purpose of testing physicochemical properties, the material has exceptionally been lyophilised in order to obtain “pure” active substance, termed “Ampholyt 20/100”. Since the a.s. as manufactured consists of 20% active matter and 80% water, the question if lyophilised “Ampholyt 20/100” or the 20% product is subjected to ecotoxicity testing is considered to be of limited relevance for the reliability of the results.
Purity	99.4 %
Composition of product	Not applicable.
Further relevant properties	The test material is a multi-component substance as specified in Section A2. A specific method of analysis was not available at the time of test performance. Therefore, TOC analysis was employed to verify concentrations.

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA 7.1

Method of analysis	TOC analysis
Preparation of TS solution for poorly soluble or volatile test substances	Not applicable.
Reference substance	No
Method of analysis for reference substance	Not applicable.
Testing procedure	
Dilution water	Synthetic freshwater as specified in Table A7.4.1.1- 22.
Test organisms	*Cyprinus carpio*, as described in Table A7.4.1.1- 23.
Test system	See Table A7.4.1.1- 24.
Test conditions	Details are presented in Table A7.4.1.1- 25 to Table A7.4.1.1- 27.
Duration of the test	96 h
Test parameter	Mortality
Sampling	Stability controls were performed at concentrations of 2, 5, and 10 mg/l at 0 h and 24 h.
Monitoring of TS concentration	Monitoring of the test substance in the test medium was not possible since the test concentrations were below the LOQ of the analytical method available at the time of test performance. Therefore, compliance of actual with nominal concentrations was assessed by (i) TOC analysis of the stock solutions and (ii) additional stability controls as specified in 0 above.
Statistics	LC_{50}: graphical interpolation; statistical analysis was not performed due to the steep dose-response curve.

Results

Limit Test	Not performed
Concentration	
Number/ percentage of animals showing adverse effects	
Nature of adverse effects	
Results test substance	
Initial concentrations of test substance	0.11, 0.19, 0.33, 0.57, 0.99 mg/l

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA 7.1

Actual concentrations of test substance	Monitoring of the test media was not possible for the reasons given in 0 above. The results of the stability controls and analyses of stock solutions performed as a substitute are presented in Table A7.4.1.1- 28. Accordingly, the test substance proved to be stable in test medium. This allows to indirectly conclude that test substance concentrations were maintained within 80% of nominal.
Effect data (mortality)	Mortality data are presented in Table A7.4.1.1- 29. Effect concentrations are reported in Table A7.4.1.1- 30.
Concentration / response curve	See Figure A7.4.1.1- 1.
Other effects	None
Results of controls	
Number/ percentage of animals showing adverse effects	None (see Table A7.4.1.1- 29).
Nature of adverse effects	Not applicable.
Test with reference substance	Not performed.
Concentrations	
Results	

Applicant's Summary and conclusion

Materials and methods	Acute toxicity of Ampholyt 20/100 to fish was tested in *Cyprinus carpio* with one control and five treatment concentrations ranging from 0.11 to 0.99 mg/l. The test was carried out according a semi-static design following the OECD guideline 203 and EC method C.1 (92/69/EEC). Monitoring of the test substance directly from the test medium was not possible since the test concentrations were below the LOQ of the analytical method available at the time of test performance. Instead, the stability of the test substance in test medium over 24 h was monitored at additional concentrations exceeding the LOQ. The test substance proved to be stable in this stability test and conformed to the nominal concentrations. Thus, it may be safely concluded that, regarding abiotic processes, the test concentrations could be maintained within 80% of nominal. However, the possibility that test substance was biologically removed by the test organisms cannot be definitely excluded. The semi-static test design (24 h renewal intervals), however, adequately ensured continuous exposure to the nominal concentrations. In conclusion it may thus be safely assumed that the criterion of maintenance of test concentrations within 80% of nominal is fulfilled.

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA 7.1

Results and discussion	Peculiarities with the analytical monitoring have been discussed in 0 above. Apart from this, the test substance does not exhibit any specific properties that may have impact on the results. No fish in the control died or showed any sub-lethal effects within a period of 96 h. Due to the steep slope of the dose-response curve statistical analysis could not be employed. The LC_{50} was therefore determined by linear interpolation.
LC_0	0.33 mg/l
LC_{50}	0.43 mg/l
LC_{100}	0.57 mg/l
Conclusion	Since the validity criteria are fulfilled (Table A7.4.1.1- 31) study is considered to be valid.
Other conclusions	
Reliability	1
Deficiencies	None

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	8th January 2013
Materials and Methods	Adopt applicants material and methods summary. Monitoring of test substance concentration not reported and explanation accepted. No limit test performed, more doses should have been tested between 0.57 and 0.99 mg/L. No light reported. Fish were fed during study.
Results and discussion	Adopt applicants version.
Conclusion	Adopt applicants version.
Reliability	3
Acceptability	Yes
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.4.1.1- 1: Preparation of TS solution for poorly soluble or volatile test substances.

Criteria	Details
Dispersion	No
Vehicle	No
Concentration of vehicle	Not applicable
Vehicle control performed	Not applicable
Other procedures	None

Table A7.4.1.1- 2: Dilution water.

Criteria	Details	
Source	Synthetic freshwater:	
	$CaCl_2 \times 2\ H_2O$:	294 mg/l
	$MgSO_4 \times 6\ H_2O$:	114 mg/l
	$NaHCO_3$:	65 mg/l
	KCl:	6 mg/l
	Sum of Ca^{2+} and Mg^{2+}:	2.5 mmol
	Ca^{2+}:Mg^{2+} ratio:	4:1
	Na^+:K^+ ratio:	10:1
Alkalinity	Not reported	
Hardness	14°dH	
pH	7.7–8.3	
Oxygen content	92–101% saturation	
Conductance	Not reported	
Holding water different from dilution water	Yes: Dechlorinated drinking water	

Table A7.4.1.1- 3: Test organisms.

Criteria	Details
Species/strain	*Cyprinus carpio*
Source	Di Mamma, Brakel, Netherlands
Wild caught	No
Age/size	3.1 cm
Kind of food	Not reported
Amount of food	2% of body mass daily
Feeding frequency	Daily
Pre-treatment	14 d acclimation
Feeding of animals during test	No

Table A7.4.1.1- 4: Test system.

Criteria	Details
Test type	Semistatic
Renewal of test solution	Daily
Volume of test vessels	10 l (refers to test medium)
Volume/animal	1 l
Number of animals/vessel	10
Number of vessels/ concentration	Not explicitly stated but according to the test design one vessel per concentration may be concluded
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.1- 5: Test conditions.

Criteria	Details		
Test temperature (°C)	Treatment	min.	max.
	Control	20.0	20.2
	0.11 mg/l	19.9	20.2
	0.19 mg/l	19.9	20.1
	0.33 mg/l	19.9	20.0
	0.57 mg/l	19.7	20.0
	0.99 mg/l	19.8	20.1
	Overall mean: 20.0°C		
Dissolved oxygen	92–101% saturation; for details see Table A7.4.1.1- 26		
pH	7.7–8.3; for details see Table A7.4.1.1- 27		
Adjustment of pH	No		
Aeration of dilution water	Yes, continuously		
Intensity of irradiation	Not reported		
Photoperiod	16:8 h (L:D)		

Table A7.4.1.1- 6: Measurements of oxygen saturation [%] during the test.

TS concentration, nominal [mg/l]	Time				
	0	**24 h (old)**	**24 h (fresh)**	**48 h**	**72 h**
Control	96	97	98	97	92
0.11	99	95	98	97	99
0.19	100	93	99	97	99
0.33	100	99	94	92	99
0.57	100	100	98	–	–
0.99	101	96	–	–	–

Table A7.4.1.1- 7: pH values in the course of the test.

TS concentration, nominal [mg/l]	Time				
	0	24 h (old)	24 h (fresh)	48 h	72 h
Control	8.0	7.8	8.0	7.9	7.8
0.11	8.1	7.8	7.9	7.9	7.8
0.19	8.2	7.8	7.9	7.9	7.7
0.33	8.2	7.7	7.8	7.8	7.8
0.57	8.2	7.8	7.8	–	–
0.99	8.3	7.8	–	–	–

Table A7.4.1.1- 8: Analytical verification of test substance concentrations.

Nominal concentration [mg/l]	Analytical values [mg/l]		Deviation [%]	
	0 h	24 h	0 h	24 h
Additional stability controls				
2	2.32	1.94	16	–16
5	5.05	490	1	–3
10	11.01	10.95	10	–1
Stock solutions				
1000	991.52	–	–0.8	–
1000	1083.78	–	8.4	–
1000	1083.57	–	8.4	–
1000	1065.50	–	6.5	–

Table A7.4.1.1- 9: Mortality data.

Test substance concentration (nominal/measured)[1] [mg/l]	Mortality							
	Number				Percentage			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Control	0	0	0	0	0	0	0	0
0.11	0	0	0	0	0	0	0	0
0.19	0	0	0	0	0	0	0	0
0.33	0	0	0	0	0	0	0	0
0.57	8	10	–	–	80	100	–	–
0.99	10	–	–	–	100	–	–	–
Temperature [°C]	See Table A7.4.1.1- 25							
pH	See Table A7.4.1.1- 27							
Oxygen [mg/l]	See Table A7.4.1.1- 26							

Table A7.4.1.1- 10: Effect data, based on nominal concentrations.

	48 h [mg/l]	95 % CI*	96 h [mg/l]	95 % CI*
LC_0	0.33	–	0.33	–
LC_{50}	0.43	0.33–0.57	0.43	0.33–0.57
LC_{100}	0.57	–	0.57	–

*) no valid confidence interval estimable due to steep dose-response curve; thus, the range of biological effect concentrations is given as a substitute

Table A7.4.1.1- 11: Validity criteria for acute fish test according to OECD Guideline 203.

	Fulfilled	Not fulfilled
Mortality of control animals <10%	☑	
Concentration of dissolved oxygen in all test vessels > 60% saturation	☑	
Concentration of test substance ≥80% of initial concentration during test	☑ *	
Criteria for poorly soluble test substances	Not applicable	

*) see discussion in 0

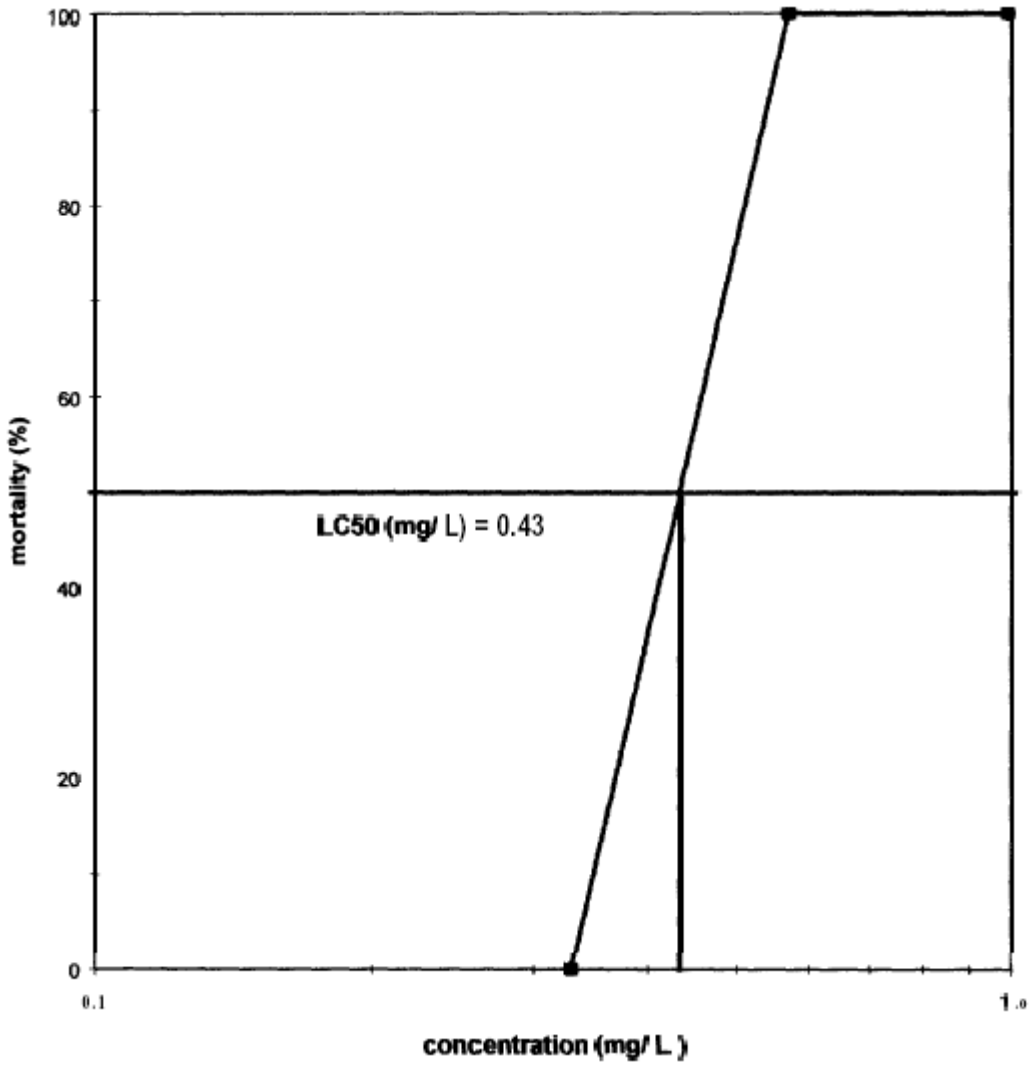


Figure A7.4.1.1- 1: Dose-response relationship after 96 h exposure to Ampholyt 20/100.

Section A7.4.1.1 **Acute toxicity to fish**
Annex Point IIA 7.1

Official use only

Reference

Reference	**A7.4.1.1/02:** [redacted] (1995): Semi-static acute toxicity test with TEGO 2000 and *Brachydanio rerio*, TNO Institute of Environmental Sciences, Delft, The Netherlands, unpublished report no.: TNO-MW.Fi94/323, June 28, 1995.
Data protection	Yes
Data owner	Goldschmidt GmbH
Companies with letter of access	No
Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

Guidelines and Quality Assurance

Guideline study	Yes OECD 203 (1992)
GLP	Yes
Deviations	No

Materials and Methods

Test material	TEGO 2000 (synonym/trade name for Ampholyt 20), please refer to Section A2. 20% a.i. (aqueous solution, “product by process”)
Lot/Batch number	490486
Specification	Ampholyt 20 as given in Section A2.
Purity	The active substance in itself is considered as pure (≥ 99%).
Composition of product	20% a.i. (aqueous solution, “product by process”)
Further relevant properties	The a.i. is a multi-component substance as specified in Section A2. A specific method of analysis was not available at the time of test performance.
Method of analysis	The actual concentrations of the a.i. of the test substance in the test solutions were not determined by chemical analysis (non-availability of a validated method). TEGO 2000 (20% a.i.) was diluted to the test concentrations: The final test was performed with six concentrations of active ingredient: 0.056, 0.10, 0.18, 0.32, 0.56, 1.0 mg a.i./l. (Appropriate test concentrations of the active ingredient were determined in a preliminary range-finding test.)

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA 7.1

Preparation of TS solution for poorly soluble or volatile test substances	Not applicable due to high solubility in water.
Reference substance	No
Method of analysis for reference substance	Not applicable.
Testing procedure	
Dilution water	DSWL, prepared from ground water (suitable for the culture of Brachydanio rerio as specified in Table A7.4.1.1- 13)
Test organisms	*Brachydanio rerio*, see Table A7.4.1.1- 14
Test system	Table A7.4.1.1- 15
Test conditions	Table A7.4.1.1- 16
Duration of the test	96 h
Test parameter	Mortality
Sampling	Survival and condition of the test fish were recorded at 0, 4, 24, 48, 72, and 96 h after start of exposure. Water parameters were measured 0, 24, 48, 72, and 96 h (if relevant) after start of the test.
Monitoring of TS concentration	Monitoring of the test substance in the test medium was not possible because it was expected that the test concentrations were below the detection limit of the analytical methods available at the time of test performance (nominal concentrations lower than 0.4 mg a.i./l). The test concentrations of the active ingredient were obtained by dilution of 50 mg TEGO 2000 in one litre of dilution water. From this solution 8.4, 15, 27, 48, 84 and 150 ml were diluted in 1.5 litre dilution water respectively, resulting in the test solutions (0.056, 0.10, 0.18, 0.32, 0.56, 1.0 mg a.i./l).
Statistics	LC_{50}, confidence interval: at a given time the mortality probability of an individual is assumed to be logistically related to the log of the test substance concentration. The variance-covariance matrix is estimated by the inverse of the information matrix.

Results

Limit Test	Not performed.
Concentration	
Number/ percentage of animals showing adverse effects	
Nature of adverse	
Results test substance	
Initial concentrations of test substance	0.056, 0.10, 0.18, 0.32, 0.56, 1.0 mg a.i./l. (nominal)

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA 7.1

Actual concentrations of test substance	Monitoring of the test media was not possible for the reasons given in 3.4.8 above. For dilution of TEGO 2000 please refer to 3.4.8 above.
Effect data (Mortality)	The mortality data are presented in Table A7.4.1.1- 17.
Concentration / response curve	Please refer to Figure A7.4.1.1- 1
Other effects	At 0.56 mg a.i./l, all fish, showed decelerated swimming at the first observation time after 4 hours. At 0.18 mg seven fish were lethargic and swimming near the bottom of the test vessel after 72 h of exposure, and two fish were additionally of dark colour.
Results of controls	
Number/ percentage of animals showing adverse effects	None (see Table A7.4.1.1- 17).
Nature of adverse effects	Not applicable
Test with reference substance	Not performed.
Concentrations	
Results	

Applicant's Summary and conclusion

Materials and methods	An acute toxicity test of TEGO 2000 to freshwater fish was performed using *Brachydanio rerio* according to OECD 203 (1992). The test was carried out under semistatic conditions with daily replacement of the test solutions and with 10 fish for the control medium and each nominal concentration tested (0.056, 0.10, 0.18, 0.32, 0.56, 1.0 mg a.i./l). The exposure duration was 96 hours.
Results and discussion	Analytical monitoring of the actual concentrations of the active ingredient was not possible. Apart from this, the test substance does not exhibit any specific properties that may have impact on the results.
LC_0	96 h: 0.1 mg a.i./l
LC_{50}	48 h: 0.24 mg a.i./l 96 h: 0.18 mg a.i./l
LC_{100}	96 h: 0.32 mg a.i./l
Conclusion	Since the validity criteria are fulfilled (Table A7.4.1.1- 31) study is considered to be valid.
Reliability	1
Deficiencies	The exact concentrations of the active ingredient of the test substance in the test solutions were not possible to determine by chemical analysis at that time.

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	8th January 2013
Materials and Methods	Adopt applications version. No concentration of the active ingredient recorded, no detection methods sensitive enough at the time.
Results and discussion	Table A7.4.1.1- 12: **Mortality data**, one reported death at 72h in 0.18mg/L a.i , no record of 9 fish?
Conclusion	Adopt applicants version
Reliability	3
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.4.1.1- 13: Dilution water.

Criteria	Details		
Source	Prepared ground water from a locality near Linschoten (the Netherlands).		
		Range finder [mmol/l]	Final test [mmol/l]
	Na^+	1.04	1.37
	K^+	0.19	0.37
	Ca^{2+}	1.35	1.28
	Mg^{2+}	0.70	0.70
	Cl^-	2.28	2.68
	SO_4^{2-}	0.61	0.72
Alkalinity	Not reported		
Hardness	205 / 198 mg/l, expressed as $CaCO_3$		
pH	8.0–8.5		
Oxygen content	Neasured in the test solution of 1.0 mg a.i./l		
Conductance	Not reported		
Holding water different from dilution water	No		

Table A7.4.1.1- 14: Test organisms.

Criteria	Details
Species/strain	*Brachydanio rerio*
Source	M.B. Ruysbroek B.V. (Noordvliet 159, Maassluis)
Wild caught	No
Age/size	Length: 2.6 ± 0.2 cm Weight:10.13 ± 0.02 g
Kind of food	Not reported
Amount of food	Not reported
Feeding frequency	–
Pre-treatment	Not reported
Feeding of animals during test	No

Table A7.4.1.1- 15: Test system.

Criteria	Details
Test type	Semistatic system
Renewal of test solution	Daily
Volume of test vessels	2 litre all-glass beakers
Volume/animal	150 ml / animal
Number of animals/vessel	10 animals /vessel
Number of vessels/ concentration	Not explicitly stated but according to the test design one vessel per concentration may be concluded
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.1- 16: Test conditions.

Criteria	Details		
Test temperature [°C]		New control medium	Control medium just before replacement
	0 h	25.2	–
	24 h	24.7	24.8
	48 h	24.5	25.2
	72 h	25.0	25.5
	96 h	–	25.4
Dissolved oxygen	Refer to Table A7.4.1.1- 18		
pH	Refer to Table A7.4.1.1- 19		
Adjustment of pH	No		
Aeration of dilution water	Yes, the control and test medium were slightly aerated.		
Intensity of irradiation	Not reported		
Photoperiod	16:8 h (L:D)		

Table A7.4.1.1- 17: Mortality data.

Test substance concentration (nominal)[1] [mga i./l]	Mortality							
	Number				Percentage			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Control	0	0	0	0	0%	0%	0%	0%
0.056	0	0	0	0	0%	0%	0%	0%
0.1	0	0	0	8	0%	0%	0%	80%
0.18	0	0	1	–	0%	0%	10%	–
0.32	0	10	–	–	0%	100%	–	–
0.56	10	–	–	–	100%	–	–	–
1.0	–	–	–	–	–	–	–	–

Table A7.4.1.1- 18: Oxygen concentrations [mg/l] in the control and test media during the test of TEGO 2000.

TS concentration, nominal [mg/l]	Oxygen concentrations				
	0	24 h (old/ fresh)	48 h (old/ fresh)	72 h (old/ fresh)	96h (old)
Control	8.1	8.4 / 7.7	8.2 / 7.4	8.1 / 7.0	7.0
0.056	8.1	8.4 / 7.7	8.2 / 7.4	8.1 / 7.2	7.3
0.1	8.1	8.4 / 7.6	8.2 / 7.5	8.1 / 7.4	7.3
0.18	8.1	8.4 / 7.7	8.2 / 7.5	8.1 / 7.5	7.0
0.32	8.1	8.4 / 7.7	– / 6.2	–	–
0.56	8.1	– / 5.9	–	–	–
1.0	8.1	–	–	–	–

Table A7.4.1.1- 19: pH values in the control and test media of TEGO 2000 during the test.

TS concentration, nominal [mg/l]	pH values				
	0	24 h (old/ fresh)	48 h (old/ fresh)	72 h (old/ fresh)	96h (old)
Control	8.1	8.1 / 7.8	8.2 / 7.9	8.2 / 7.7	7.8
0.056	8.0	8.1 / 7.8	8.2 / 7.9	8.2 / 7.8	7.9
0.1	8.1	8.1 / 7.8	8.2 / 7.9	8.2 / 7.8	7.9
0.18	8.1	8.1 / 7.8	8.2 / 8.0	8.2 / 7.9	7.8
0.32	8.1	8.1 / 7.9	– / 7.8	–	–
0.56	8.1	– / 7.7	–	–	–
1.0	8.1	–	–	–	–

Table A7.4.1.1- 20: Effect data.

	48 h [mg/l][1]	95 % CI	96 h [mg/l][1]	95 % CI
LC_0	0.18		0.1	
LC_{50}	0.24	0.18–0.32	0.18	0.12–0.18
LC_{100}	0.32		0.32	

[1]) no valid confidence interval estimable due to steep dose-response curve; thus, the range of biological effect concentrations is given as a substitute

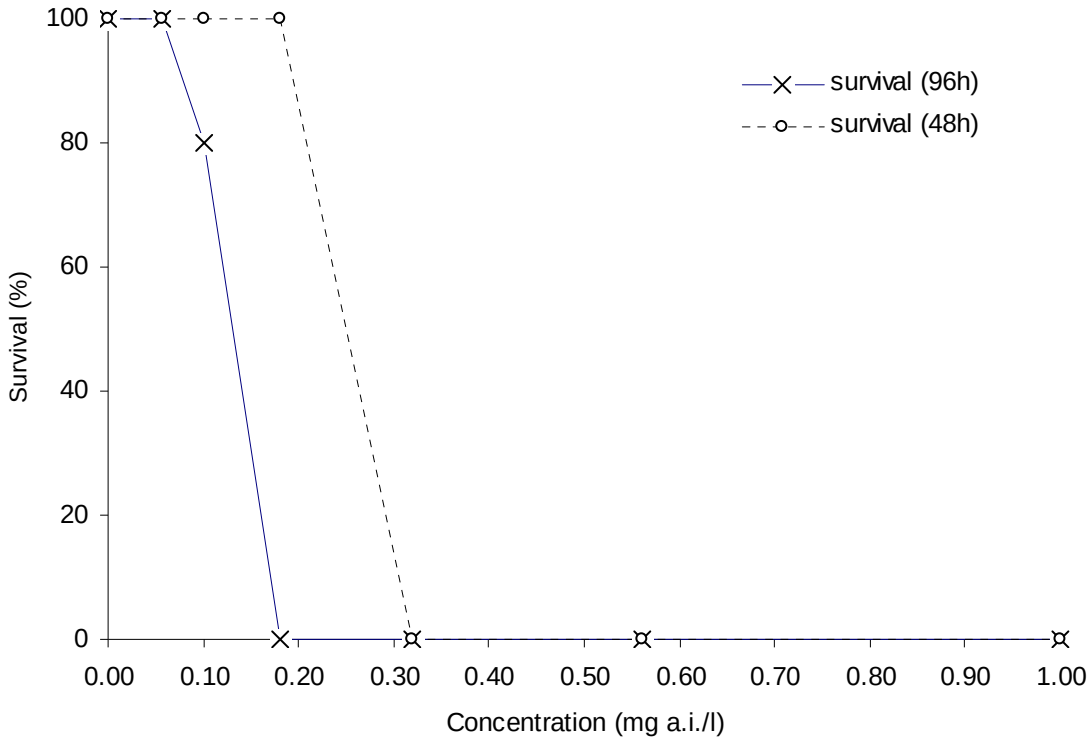


Figure A7.4.1.1- 1: Concentration-response relationship after 96 or 48 h exposure to TEGO2000.

Table A7.4.1.1- 21: Validity criteria for acute fish test according to OECD Guideline 203.

	Fulfilled	Not fulfilled
Mortality of control animals <10%	☑	
Concentration of dissolved oxygen in all test vessels > 60% saturation	☑	
Concentration of test substance ≥80% of initial concentration during test	☑	
Criteria for poorly soluble test substances	Not applicable	

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Reference

Reference	**A7.4.1.1/03:** [redacted] (2008) *Oncorhynchus mykiss*, acute toxicity test (OECD 203) flow-through exposure – effect of Ampholyt 20 on the acute toxicity to rainbow trout. Fraunhofer-Institute for Molecular Biology and Applied Ecology (IME), Schmallenberg, Germany, Report no. EBR-013/4-13, March 20, 2008 (unpublished).
Data protection	Yes
Data owner	Goldschmidt GmbH
Companies with letter of access	No
Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

Guidelines and Quality Assurance

Guideline study	Yes EC C.1 (92/69/EEC) OECD 203 (1992)
GLP	Yes
Deviations	No

Materials and Methods

Test material	Ampholyt 20 as given in Section A2.
Lot/Batch number	ES67345616
Specification	Ampholyt 20 as given in Section A2. The active substance as manufactured is obtained by the production process as a “product-by-process”, constituting a 20% (w/w) aqueous solution of the active matter.
Purity	99 % w/w
Composition of product	Not applicable
Further relevant properties	The test material is a multi-component substance as specified in Section A2. Thus, analytical verification of test substance concentrations employed a lead substance concept, focussing on the C_{12}-alkyl compounds only.

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA 7.1

Method of analysis	The test item concentrations were analysed using HPLC-MS/MS. The limit of quantification for each lead compound was 0.1 µg/L. To assess the concentration of the test item Ampholyt 20, four "lead components" of the mixture were analysed, accounting together for 65.92 % of total active substance. The concentrations of the single lead compounds were summed up and extrapolated to the total active substance concentration. The details are summarised in Section A4.2.
Preparation of TS solution for poorly soluble or volatile test substances	Not applicable.
Reference substance	No
Method of analysis for reference substance	Not applicable.
Testing procedure	
Dilution water	Specified in Table A7.4.1.1- 22
Test organisms	Table A7.4.1.1- 23
Test system	Table A7.4.1.1- 24
Test conditions	Table A7.4.1.1- 25
Duration of the test	96 h
Test parameter	Mortality
Sampling	Analytical verification was performed for the nominal test concentrations 150, 300, and 600 and 1200 µg a.s. per litre at test start, and for 150, 300, and 600 µg/L at 48 h and at test end.
Monitoring of TS concentration	Since the measured test item concentrations deviated by more than 20% from the nominal concentrations further samples were analysed in order to enable calculation of effect values based on mean measured concentrations.
Statistics	All statistical calculations were based on mean measured concentrations of the active substance of Ampholyt 20. Calculations were performed with the computer software ToxRat Professional version 2.09 (release 08.11.2006) by ToxRat® Solutions GmbH. When the test results showed a concentration-response relationship, the data were analysed by regression to determine the EC50 including the 95% confidence interval as well as the EC10 using Probit-analysis assuming log-normal distribution of the values.

Results

Limit Test	Not performed
Concentration	–
Number/ percentage of animals showing adverse effects	–
Nature of adverse effects	–

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA 7.1

Results test substance	
Initial concentrations of test substance	Nominal concentrations of 93.5, 188, 375, 750, 1500, 3000, and 6000 µg Ampholyt 20 per litre, representing 18.8, 37.5, 75, 150, 300, 600, and 1200 µg active substance (a.s.) per litre.
Actual concentrations of test substance	In the test media the mean measured concentrations of the test item were in the range of 43 % to 57 % of nominal, independent of the concentration. For the evaluation of the effect concentrations the means of the measured four highest concentrations were used. The means of the treatments were calculated to be 84.9, 136.3, 258.6, and 672.5 µg a.s./L (56.6 %, 45.4 %, 43.1 %, and 56.1 % of nominal).
Effect data (mortality)	At concentrations up to and including 84.9 µg a.s./L, no mortality was observed. At 136.3 µg a.s./L, there was no effect during the first three days of the test. On the last day, three of ten fish died. At 258.6 µg a.s./L, mortality started on the second day. At the highest concentration all fish died within 24 h. Please refer to Table A7.4.1.1- 29 and Table A7.4.1.1- 30.
Concentration / response curve	There was a clear concentration and time-effect dependency. Please refer to Figure A7.4.1.1- 2.
Other effects	
Results of controls	None of the introduced control animals died.
Number/ percentage of animals showing adverse effects	Please refer to Table A7.4.1.1- 29.
Nature of adverse effects	At concentrations up to and including 84.9 µg a.s./L, no abnormal condition or behavior was observed. At 136.3 µg a.s./L, there was no effect during the first three days of the test. On the last day, the surviving fish exhibited abnormal behavior characterized by slow or uncoordinated swimming at the water surface and/or dark discoloration. At 258.6 µg a.s./L, similar effects already started during the first day. At the highest concentration, toxic effects started immediately.
Test with reference substance	–
Concentrations	–
Results	–

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA 7.1

Applicant's Summary and conclusion

Materials and methods	The influence of Ampholyt 20 on acute toxicity to rainbow trout (*Oncorhynchus mykiss*) was investigated in accordance with the OECD Guideline 203 and EU method C.1 (92/69/EEC). The fish were placed in water containing the test item at nominal concentrations of 93.5, 188, 375, 750, 1500, 3000, and 6000 µg test item per litre, representing 18.8, 37.5, 75, 150, 300, 600, and 1200 µg active substance (a.s.) per litre. The test was conducted under flow-through conditions for 96 hours. Effects on survival were determined after 24, 48, 72, and 96 hours. Samples of test solutions were taken at test start, after 48 hours and at test end. In test media the mean measured concentrations of the test item were in the range of 43 % to 57 % of nominal, independent of the concentration. For the evaluation of the effect concentrations the means of the measured four highest concentrations were used. At nominal concentrations up to and including 84.9 µg a.s./L, neither mortality nor abnormal condition or behavior was observed. At higher concentrations, there was a clear concentration and time-effect dependency, starting with 30 % mortality at 136.3 µg a.s./L on day four and ending with 100 % mortality at 672.5 µg a.s./L on the first day. Clinical signs of intoxication were apparent only in surviving or moribund fish at concentrations causing partial or total mortality.
Results and discussion	
LC_0	48 h: 84.9 µg a.s./L 96 h: 84.9 µg a.s./L
LC_{10}	48 h: 253.3 µg a.s./L 96 h: 109.5 µg a.s./L
LC_{50} (95% CL)	48 h: 351.0 µg a.s./L 96 h: 207.4 µg a.s./L (CL: 157.5–273.2 µg a.s./L)
Conclusion	Since the validity criteria are fulfilled – except maintenance of test concentrations within 20 % initial, which is however addressed by consideration of measured concentrations (Table A7.4.1.1- 31) – the study is considered to be valid.
Other conclusions	
Reliability	1
Deficiencies	None

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	10th January 2013
Materials and Methods	Adopt applicants summary.
Results and discussion	Adopt applicants summary. Measured values to be used in results as described by applicant.
Conclusion	Acceptable.
Reliability	1
Acceptability	Acceptable.
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.4.1.1- 22: Dilution water.

Criteria	Details
Source	Cu-free tap water, Fraunhofer IME
Alkalinity	0.7–1.1 mmol/L
Total Hardness	0.7–1.0 mmol/L
pH	At test start: 8.0–8.1, for details see Table A7.4.1.1- 27
Oxygen content	at test start: 80–91 % saturation, for details see Table A7.4.1.1- 26
Conductance	161.3–183.7
Holding water different from dilution water	No

Table A7.4.1.1- 23: Test organisms.

Criteria	Details
Species/strain	*Oncorhynchus mykiss* (Walbaum) (Teleostei, Salmonidae, Salmoniformes)
Source	NRW Landesanstalt für Fischerei (governmental fisheries agency), Albaum, Germany on March 22, 2007 and further bred in the test facility.
Wild caught	No
Age/size	5 ± 1cm
Kind of food	Acc. to the guidelines, the fish were not fed during the test.
Amount of food	n.a.
Feeding frequency	n.a.
Pre-treatment	Not reported, the fish were bred in the test facility.
Feeding of animals during test	No

Table A7.4.1.1- 24: Test system.

Criteria	Details
Test type	Flow-through system
Renewal of test solution	n.a., flow-through rate 2.5 L/h (daily turnover: 5 vol)
Volume of test vessels	42 × 28 × 28 cm, approx. 25 L test solution
Volume/animal	2.5 L/animal
Number of animals/vessel	10
Number of vessels/ concentration	1
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.1- 25: Test conditions.

Criteria	Details
Test temperature (°C)	Test start: 13.5 °C 24 h: 13.6 °C 48 h: 13.7 °C 72 h: 13.6 °C 96 h: 13.8 °C
Dissolved oxygen	92–101% saturation; for details see Table A7.4.1.1- 26
pH	7.7–8.3; for details see Table A7.4.1.1- 27
Adjustment of pH	No
Aeration of dilution water	Yes, continuously
Intensity of irradiation	Not reported
Photoperiod	16:8 h (L:D)

Table A7.4.1.1- 26: Measurements of oxygen saturation [%] of the test media during the test. a.s. = active substance, Concentrations given as nominal concentrations.

Nominal a.s. concentration (µg/L)	Test duration							
	0 h	24 h	48 h	72 h	96 h	Mean	Min	Max
Control	80	76	70	78	82	77	70	82
18.8	87	95	86	89	89	89	86	95
37.5	84	89	86	88	89	87	84	89
75	84	88	84	88	89	87	84	89
150	84	92	82	88	88	87	82	92
300	84	89	86	94	89	88	84	94
600	88	80	85	87	82	84	80	88
1200	91	91	–	–	–	91	91	91

- no measurement since all fish died after 24 hours

Table A7.4.1.1- 27: pH values in the course of the test.

Nominal a.s. concentration (µg/L)	Test duration							
	0 h	24 h	48 h	72 h	96 h	Mean	Min	Max
Control	8.0	8.0	8.0	8.0	7.9	8.0	7.9	8.0
18.8	8.1	8.0	8.0	7.9	8.1	8.0	7.9	8.1
37.5	8.1	8.0	8.0	7.9	8.1	8.0	7.9	8.1
75	8.1	8.0	7.9	7.9	8.1	8.0	7.9	8.1
150	8.0	8.0	7.9	7.9	8.0	8.0	7.9	8.0
300	8.1	8.0	8.0	7.9	8.0	8.0	7.9	8.1
600	8.1	7.7	7.8	7.8	8.0	7.9	7.7	8.1
1200	8.1	7.9	–	–	–	8.0	7.9	8.1

– no measurement since all fish died after 24 hours

Table A7.4.1.1- 28: Measured concentrations and mean of the four "lead components" [redacted] of the active substance Ampholyt 20 in the test media.

Nominal concentration			Measured concentrations of Ampholyt 20 [µg a.s./L]			
Total a.s. [µg/L]	**Sum of lead components**		**Test start**	**48 h**	**96 h**	**Mean**
150	98.9	µg/L	66.3	41.0	60.5	56.0
		% nom.	67.0	41.5	61.2	56.6
300	197.8	µg/L	119.1	91.5	59.0	89.9
		% nom.	60.2	46.3	29.8	45.4
600	395.5	µg/L	185.2	198.1	128.1	170.5
		% nom.	46.8	50.1	32.4	43.1
1200	791.0	µg/L	443.3	n.a.	n.a.	443.3
		% nom.	56.0			56.0
	Extrapolated to total a.s.					
150	150	µg/L				84.9
		% nom.				56.6
300	300	µg/L				136.3
		% nom.				45.4
600	600	µg/L				258.6
		% nom.				43.1
1200	1200	µg/L				672.5
		% nom.				56.1

Table A7.4.1.1- 29: Cumulative mortality and clinical signs of intoxication during the test period of 96 h (n per vessel = 10). a.s. = active substance; m m. = mean measured; Concentrations are given as nominal and mean measured concentrations.

Test substance concentration (µg/L)	Test duration				
	8 h	24 h	48 h	72 h	96h
Control	0	0	0	0	0
18.8 n.	0	0	0	0	0
37.5 n.	0	0	0	0	0
75 n.	0	0	0	0	0
150 n. (84.9 m.m.)	0	0	0	0	0
300 n. (136.3 m.m.)	0	0	0	0	3[msd]
600 n. (258.6 m.m.)	0	0[md]	1[mpd]	3[mpsbd]	6[msbd]
1200 n. (672.5 m.m.)	4[upb]	10	10	10	10
Temperature [°C]	See Table A7.4.1.1- 25				
pH	See Table A7.4.1.1- 27				
Oxygen [mg/l]	See Table A7.4.1.1- 26				

More than one surviving fish showed the following clinical signs of intoxication:

m = slow movements

u = uncoordinated swimming

p = unbalanced position

s = swimming at the water surface

b = lying at the bottom of the test vessel

d = dark colour

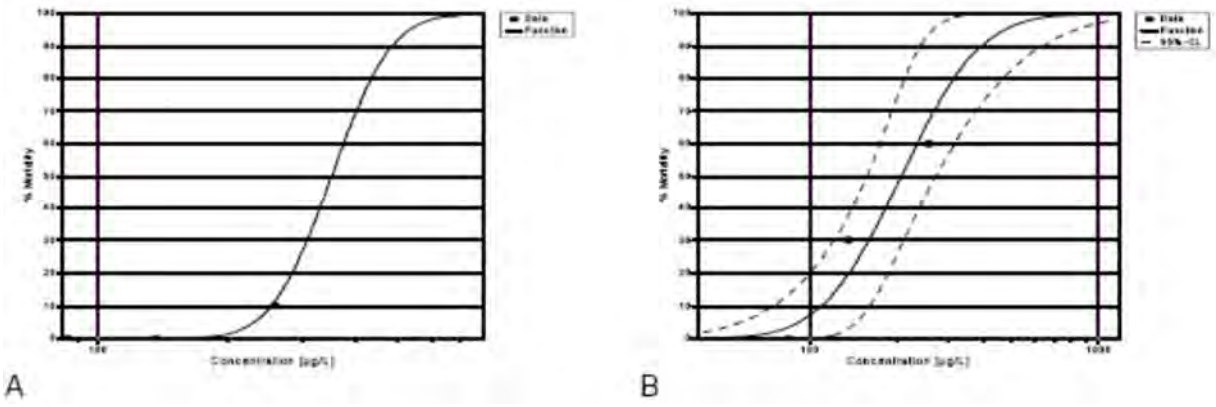

Figure A7.4.1.1- 2: Effects on mortality of the introduced *Oncorhynchus mykiss* as observed after 48 h and 96 h. A: Concentration-effect curve after 48 h. No calculation of confidence levels (CL) possible; B: Concentration-effect curve after 96 h. No mortality was found up to a nominal concentration of 150 µg a.s./L (84.9 µg/L mean measured). Thus, the lower treatments (nominal 18.8, 37.5 and 75 µg a.s./L) were excluded from the probit analysis.

Table A7.4.1.1- 30: Effect data, LC values after 48 h and 96 h [µg a.s./L] LC = Lethal concentration; CL = Confidence limits; n.d. = not determined due to mathematical reasons; effect concentrations given as mean measured concentrations.

	48 h [µg/l]	96 h [µg/l]
LC_0	84.9	84.9
LC_{10}	253.3	109.5
LC_{50} (95% CL)	351.0 (n.d.)	207.4 (157.5–273.2)

Table A7.4.1.1- 31: Validity criteria for acute fish test according to OECD Guideline 203.

	Fulfilled	Not fulfilled
Mortality of control animals <10%	☑	
Concentration of dissolved oxygen in all test vessels > 60% saturation	☑	
Concentration of test substance ≥80% of initial concentration during test		☑ *
Criteria for poorly soluble test substances	Not applicable	

*) The results were therefore evaluated based on measured concentrations

Section A7.4.1.2 **Acute toxicity to invertebrates**
Annex Point IIA 7.2

Official use only

Reference

Reference	**A7.4.1.2/01:** [redacted] (2002) Ampholyt 20/100 – determination of the immobilisation of *Daphnia magna* Straus (acute immobilisation test), Infracor GmbH, Marl, Germany, report no. DK 795, October 01, 2002 (unpublished).
Data protection	Yes
Data owner	Goldschmidt GmbH
Companies with letter of access	No
Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

Guidelines and Quality Assurance

Guideline study	Yes EC C.2 (92/69/EEC) OECD 202 (I) (1984)
GLP	Yes
Deviations	No

Materials and Methods

Test material	Ampholyt 20/100 as given in Section A2.
Lot/Batch number	ES62403356
Specification	As given in Section A2. The active substance as manufactured is obtained by the production process as a “product-by-process”, constituting a 20% (w/w) aqueous solution of the active matter. For the purpose of testing physicochemical properties, the material has exceptionally been lyophilised in order to obtain “pure” active substance, termed “Ampholyt 20/100”. Since the a.s. as manufactured consists of 20% active matter and 80% water, the question if lyophilised “Ampholyt 20/100” or the 20% product is subjected to ecotoxicity testing is considered to be of limited relevance for the reliability of the results.
Purity	99.4%
Composition of product	Not applicable
Further relevant properties	The test material is a multi-component substance as specified in Section A2. A specific method of analysis was not available at the time of test performance. Therefore, TOC analysis was employed to verify concentrations.

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA 7.2

Method of analysis	TOC analysis
Preparation of TS solution for poorly soluble or volatile test substances	Not applicable
Reference substance	Potassium dichromate
Method of analysis for reference substance	Not stated.
Testing procedure	
Dilution water	According to guideline, for details see Table A7.4.1.2- 2.
Test organisms	*Daphnia magna*, as described in Table A7.4.1.2- 3.
Test system	See Table A7.4.1.2-4.
Test conditions	Details are given in Table A7.4.1.2-5.
Duration of the test	48 h
Test parameter	Immobility
Sampling	The test solution itself was not sampled since test concentrations were below the LoQ of the analytical method (TOC analysis). Instead, a stability control with the stock solution (1000 mg/l, nominal), and TS solutions concentrated 2, 5, and 10 mg/l was performed in parallel. Samples for analysis were drawn at 0 and 48 h.
Monitoring of TS concentration	Monitoring of the test substance in the test medium was not possible since the test concentrations were below the LOQ of the analytical method available at the time of test performance. Therefore, compliance of actual with nominal concentrations was assessed by (i) TOC analysis of the stock solutions and (ii) additional stability controls as specified in 0 above.
Statistics	EC_{50}, graphically (due to steep dose-response curve).

Results

Limit Test	Not performed.
Concentration	
Number/ percentage of animals showing adverse effects	
Nature of adverse effects	
Results test substance	
Initial concentrations of test substance	0.08, 0.14, 0.24, 0.42, 0.72, 1.2 mg/l
Actual concentrations of test substance	In the stability controls, TS concentrations were maintained within 80% of nominal. Details are presented in Table A7.4.1.2- 6.
Effect data (Immobilisation)	See Table A7.4.1.2-7.

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA 7.2

Concentration / response curve	A graphical presentation is given in Figure A7.4.1.2- 1.
Other effects	None
Results of controls	No effects, see Table A7.4.1.2-7.
Test with reference substance	Conducted quarterly in the performing laboratory.
Concentrations	1.0 and 2.0 mg/l
Results	c [mg/l] % immobilised *Daphnia* after 24 h 1.0 35 2.0 100 Thus, the 24-h EC_{50} may be expected to be in close agreement with the mean value from the EEC ring-test (1.5 mg/l).

Applicant's Summary and conclusion

Materials and methods	The acute toxicity of Ampholyt 20/100 to aquatic invertebrates was tested in *Daphnia magna* with one control and six test concentrations ranging from 0.08 to 1.2 mg/l. The test was carried out according to the OECD guideline 202 and EC method C.2 (92/69/EEC). Maintenance of nominal test concentrations was monitored by a parallel stability control as described under 0 above. Accordingly, it can be safely assumed that actual test concentrations were within 80% of nominal. No deviations from the method prescribed by the guideline were reported.
Results and discussion	Due to the steep dose-response curve, the EC_{50} had to be determined graphically and a confidence interval cannot be given. The test substance is not known to exhibit any properties that could have affected the outcome of the test.
EC_0	0.08 mg/l
EC_{50}	0.11 mg/l
EC_{100}	0.14 mg/l
Conclusion	All validity criteria were fulfilled (see Table A7.4.1.2-9). Thus, the study is considered to be valid without restrictions.
Reliability	1
Deficiencies	None

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	10th January 2013
Materials and Methods	No limit test performed. If performed testing would have been at smaller concentrations.
Results and discussion	The LC_{50} reported from nominal values in this study is not as accurate as measured values, even after only 24 hrs. The stability studies on high concentrations help, but the result is not as reliable as if measured values given.
Conclusion	Study acceptable but with restrictions.
Reliability	3
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.4.1.2- 1: Preparation of TS solution for poorly soluble or volatile test substances.

Criteria	Details
Dispersion	Not applicable
Vehicle	Not applicable
Concentration of vehicle	Not applicable
Vehicle control performed	Not applicable
Other procedures	Not applicable

Table A7.4.1.2- 2: Dilution water.

Criteria	Details
Source	Reconstituted water
Alkalinity	Not stated
Hardness	14° dH (= 250 mg $CaCO_3$/l)
pH	7.8–8.1
Ca / Mg ratio	4:1
Na / K ratio	10:1
Oxygen content	8.2 mg/l
Conductance	Not stated
Holding water different from dilution water	Not stated

Table A7.4.1.2- 3: Test organisms.

Criteria	Details
Species/strain	*Daphnia magna*, clone 5
Source	Received from Bayer AG, Leverkusen, in 1991, subsequently bred in the performing laboratory
Age	< 24 h
Breeding method	In 1-L beakers, using “M4 medium”
Kind of food	*Desmodesmus subspicatus*
Amount of food	In equilibrium with the daphnids’ consumption rate
Feeding frequency	Daily
Pre-treatment	None
Feeding of animals during test	No

Table A7.4.1.2-4: Test system.

Criteria	Details
Renewal of test solution	None
Volume of test vessels	10 ml (test solution volume)
Volume/animal	2 ml
Number of animals/vessel	5
Number of vessels/ concentration	4
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.2-5: Test conditions.

Criteria	Details		
Test temperature	20.21°C (range: 20.04–20.32°C)		
Dissolved oxygen [mg/l]	TS conc. [mg/l]	0 h	48 h
	Control	8.2	8.6
	0.08	8.3	7.8
	0.14	8.3	7.9
	0.24	8.3	7.8
	0.42	8.3	7.7
	0.72	8.3	7.6
	1.2	8.3	7.8
pH	TS conc. [mg/l]	0 h	48 h
	Control	8.1	7.9
	0.08	8.1	7.8
	0.14	8.1	7.8
	0.24	8.1	7.8
	0.42	8.1	7.8
	0.72	8.1	7.8
	1.2	8.1	7.8
Adjustment of pH	No		
Aeration of dilution water	No		
Quality/Intensity of irradiation	Not applicable		
Photoperiod	24 h dark		

Table A7.4.1.2- 6: Analytical verification of test substance concentrations.

Nominal concentration [mg/l]	Analytical values [mg/l]		Deviation [%]	
	0 h	**24 h**	**0 h**	**24 h**
Additional stability controls				
2	2.32	1.98	16	–15
5	5.05	4.50	1	–11
10	11.01	9.77	10	–11
Stock solutions				
10	10.26	9.91	3	–3
1000	1083.78	–	8.4	–

Table A7.4.1.2-7: Immobilisation data.

Test substance concentration (nominal) [mg/l]	**Immobile *Daphnia***			
	Number		**Percentage**	
	24 h	**48 h**	**24 h**	**48 h**
Control	0	0	0	0
0.08	0	0	0	0
0.14	2	20	10	100
0.24	20	20	100	100
0.42	20	20	100	100
0.72	20	20	100	100
1.2	20	20	100	100

For pH, temperature and oxygen see Table A7.4.1.2-5 above

Table A7.4.1.2-8: Effect data.

	EC_{50} (nominal)	**95 % CI***	**EC_0 (nominal)**	**EC_{100} (nominal)**
24 h [mg/l]	0.18	–	0.08	0.24
48 h [mg/l]	0.11	–	0.08	0.14

*) due to the steep dose-response curve, no meaningful confidence interval estimates could be obtained

Table A7.4.1.2-9: Validity criteria for acute *Daphnia* immobilisation test according to OECD Guideline 202.

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

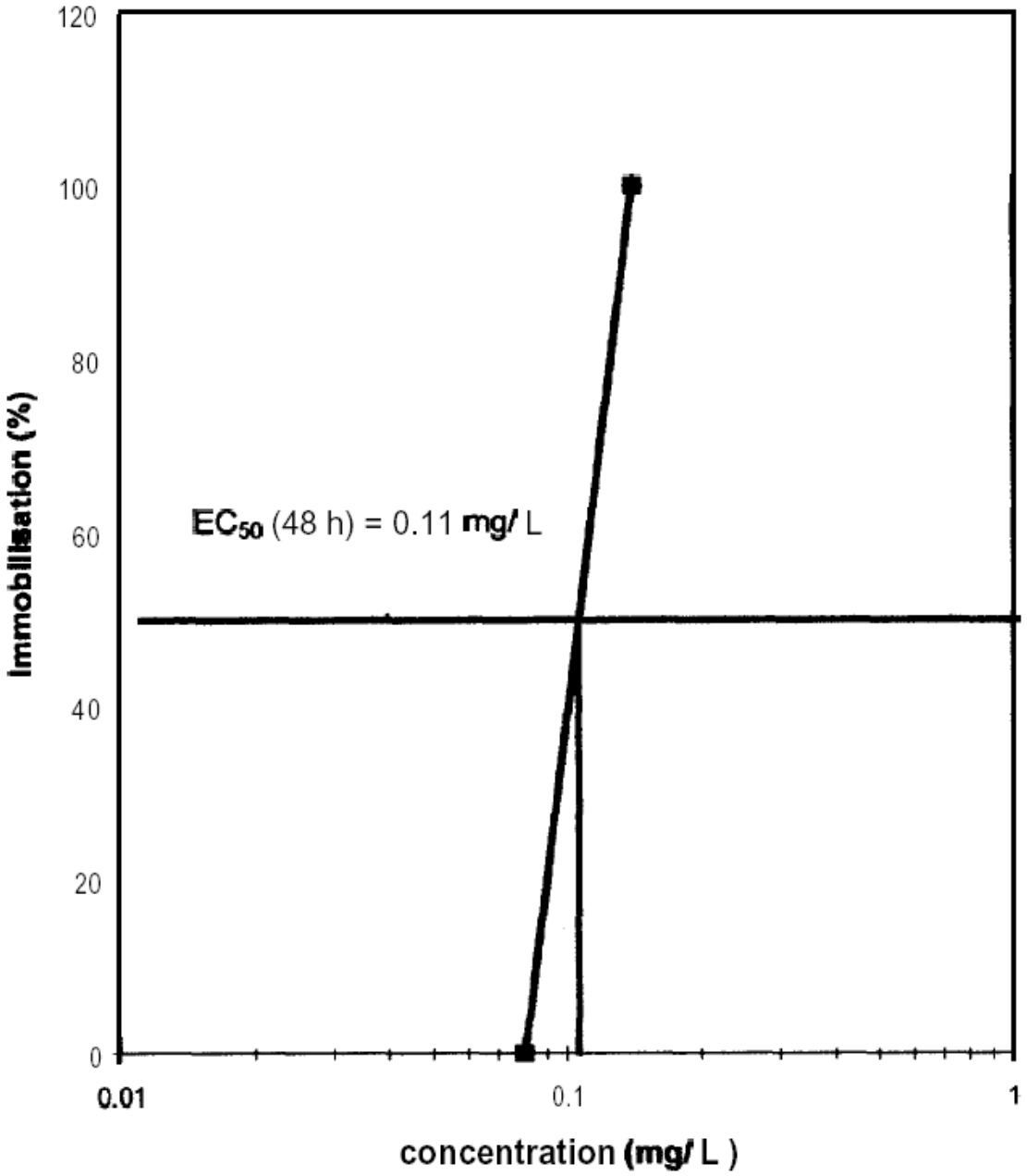

Figure A7.4.1.2- 1: Concentration-response curve (after 48 h) for the acute toxicity of Ampholyt 20/100 to *Daphnia magna*.

Section A7.4.1.2 **Acute toxicity to invertebrates**
Annex Point IIA 7.2

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Reference

Reference	**A7.4.1.2/02:** [redacted] (1995): Static acute toxicity test with TEGO 2000 and Daphnia magna, TNO Institute of Environmental Sciences, Delft, The Netherlands, unpublished report no. IMW-94-0039-0, June 28, 1995.
Data protection	Yes
Data owner	Goldschmidt GmbH
Companies with letter of access	No
Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

Guidelines and Quality Assurance

Guideline study	Yes OECD 202 (I) (1984).
GLP	Yes
Deviations	No

Materials and Methods

Test material	TEGO 2000 (synonym/trade name for Ampholyt 20), please refer to Section A2. 20% a.i. (aqueous solution, “product by process”)
Lot/Batch number	490486
Specification	Ampholyt 20 as given in Section A2.
Purity	The active substance in itself is considered as pure (≥ 99%).
Composition of product	20% a.i. (aqueous solution, “product by process”)
Further relevant properties	The a.i. is a multi-component substance as specified in Section A2. A specific method of analysis was not available at the time of test performance.
Method of analysis	The actual concentrations of the a.i. of the test substance in the test solutions were not determined by chemical analysis (non-availability of a validated method). TEGO 2000 (20% a.i.) was diluted to the test concentrations: The final test was performed with six concentrations: 0.010, 0.018, 0.032, 0.056, 0.1, and 0.18 mg a.i./l. (Appropriate test concentrations of the active ingredient were determined in a preliminary range-finding test.)

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA 7.2

Preparation of TS solution for poorly soluble or volatile test substances	Not applicable due to high solubility in water.
Reference substance	No
Method of analysis for reference substance	Not applicable.
Testing procedure	
Dilution water	DSWL, prepared from ground water (suitable for the culture of *Daphnia magna*).
Test organisms	*Daphnia magna*, as described in Table A7.4.1.2- 11.
Test system	See Table A7.4.1.2-12.
Test conditions	Please refer to Table A7.4.1.2-13.
Duration of the test	48 h
Test parameters	Immobility
Sampling	Not performed
Monitoring of TS concentration	Monitoring of the test substance in the test medium was not possible because the test concentrations were expected to be below the detection limit of the analytical methods available at the time of test performance (nominal concentrations lower than 0.4 mg a.i./l).
Statistics	EC_{50} by logistic regression.

Results

Limit Test	Not performed
Concentration	
Number/ percentage of animals showing adverse effects	
Nature of adverse	
Results test substance	
Initial concentrations of test substance	The test concentrations of the active ingredient were obtained by dilution of 50 mg TEGO 2000 in one litre of dilution water. From this solution, 1.0, 1.8, 3.2, 5.6, 10 and 18 ml were diluted with one litre of dilution water, resulting in the test solutions of 0.010, 0.018, 0.032, 0.056, 0.1, and 0.18 mg a.i./l.
Actual concentrations of test substance	The concentrations of test substance could not be determined due to non-availability of a suitable analytical method.
Effect data (Immobilisation)	Effect data are given in Table A7.4.1.2-16.
Concentration / response curve	A graph of the effect after 48 h exposure is presented in Figure A7.4.1.2- 2.

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA 7.2

Other effects	At 0.1 mg a.i./l, one daphnid was mobile but swam slower and with irregular movements. This observation was also made in 14 animals exposed to 0.056 mg a.i. after 48 h.
Results of controls	None of the *Daphnia* in the control test vessels was immobile after 48 h.
Test with reference substance	Not performed.
Concentrations	
Results	

Applicant's Summary and conclusion

Materials and methods	The acute toxicity of TEGO 2000 to aquatic invertebrates was tested in *Daphnia magna* with one control and six test concentrations ranging from 0.01 to 0.18 mg/l. The test was carried out according to the OECD guideline 202. No deviations from the method prescribed by the guideline were reported except the omitted quantification of the actual concentration of a.i. in the test solutions due the non-availability of a validated analytical method at that time.
Results and discussion	Regardless of the exact quantification of the a.i., the study is considered to be valid.
EC_0	EC_0 (48 h) = 0.032 mg a.i./l
EC_{50}	EC_{50} (24 h) = 0.11 mg a.i./l (95% CI = 0.1–0.12) EC_{50} (48 h) = 0.06 mg a.i./l (95% CI = 0.01–0.07)
EC_{100}	EC_{100} (48 h) = 0.18 mg a.i./l
Conclusion	
Other conclusions	
Reliability	1
Deficiencies	None

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	10th January 2013
Materials and Methods	No stability study performed to determine reliability of nominal values. No limit test, although results of study A7-4-1-2/01 serves as the limit test.
Results and discussion	Accept applicant's summary although actual LC_{50} results may be unreliable as nominal values reported.
Conclusion	Acceptable.
Reliability	3
Acceptability	Acceptable.
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.4.1.2- 10: Dilution water.

Criteria	Details		
Source	Prepared ground water from a locality near Linschoten (the Netherlands) (test medium)		
		Range finder [mmol/l]	Final test [mmol/l]
	Na^+	1.04	1.37
	K^+	0.19	0.37
	Ca^{2+}	1.35	1.28
	Mg^{2+}	0.70	0.70
	Cl^-	2.28	2.68
	SO_4^{2-}	0.61	0.72
Alkalinity	Not reported		
Hardness	205/198 mg/l, expressed as $CaCO_3$		
pH	8.0–8.5		
Oxygen content	Measured in the test solution of 1.0 mg a.i./l, see Table A7.4.1.2-14		
Conductance	Not reported		
Holding water different from dilution water	No		

Table A7.4.1.2- 11: Test organisms.

Criteria	Details
Species/strain	*Daphnia magna*
Source	TNO (cultured in the laboratory since about 1967, at least the last 10 years cultured in the dilution water, see Table A7.4.1.2- 10)
Age	All test organisms are of the same age (less than 24 h)
Breeding method	Every week cultures are started with ca. 125 Daphnia of the same age (~1 day) in ca. 4 litres of dilution water (20 °C, 16:8 h [light:dark]). At least once a week the medium is completely replaced and at the same time all young born are removed. After 4 weeks the cultures are discarded.
Kind of food	Algal cells (*Chlorella*), yeast
Amount of food	4×10^9 algal cells and 0.13 g yeast per 4 l of culture
Feeding frequency	Daily
Pretreatment	Not reported
Feeding of animals during test	No

Table A7.4.1.2-12: Test system.

Criteria	Details
Renewal of test solution	No
Volume of test vessels	150 ml
Volume/animal	100 ml / 5 animals
Number of animals/vessel	5
Number of vessels/ concentration	4
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.2-13: Test conditions.

Criteria	Details
Test temperature	The temperature was measured in one of the control media: 20.3 °C and 19.9 °C at the beginning and at the end of the test respectively.
Dissolved oxygen	Oxygen concentrations are given in Table A7.4.1.2-14
pH	See Table A7.4.1.2-15
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	Not performed
Photoperiod	16:8 h [light:dark] with transition periods of ca. 30 min

Table A7.4.1.2-14: Oxygen concentrations (mg/l) in the control and test media during the test of TEGO 2000 (4 replicates/concentration).

TS concentration, nominal [mg/l]	Oxygen concentrations (mg/l)				
	0 h	48 h (24 h for the values at TS concentration 0.18)			
Control	8.6	8.1	8.0	7.9	7.7
0.010	8.6	6.5	6.7	7.1	7.2
0.018	8.6	7.6	7.5	7.6	7.6
0.032	8.7*	7.2	7.3	6.5	6.5
0.056	8.7*	6.9	7.5	7.9	6.2
0.1	8.7*	6.6	7.4	7.1	6.5
0.18	8.7	9.3	9.2	9.3	9.3

*) in the study report, the values are hardly to decode

Table A7.4.1.2-15: pH-values in the control and test media during the test (4 replicates/concentration)

TS concentration, nominal [mg/l]	pH values				
	0 h	48 h (24 h for the values at TS concentration 0.18)			
Control	8.1	7.9	7.9	7.9	7.8
0.010	8.1	7.8	7.8	7.8	7.8
0.018	8.1	7.9	7.9	7.9	7.8
0.032	8.1	7.8	7.8	7.8	7.8
0.056	8.1	7.8	7.8	7.9	7.8
0.1	8.1	7.8	7.9	7.8	7.7
0.18	8.1	8.3*	8.2*	8.2*	8.2*

*) in the study report, the values are hardly to decode

Table A7.4.1.2-16: Immobilisation data.

Test substance concentration (nominal) [mg a.i./l]	Mobile *Daphnia*			
	Number		Percentage	
	24 h	48 h	24 h	48 h
Control	20	20	100	100
0.010	20	20	100	100
0.018	20	20	100	100
0.032	20	20	100	100
0.056	20	14	100	70
0.1	13	1	65	5
0.18	0	0	0	0

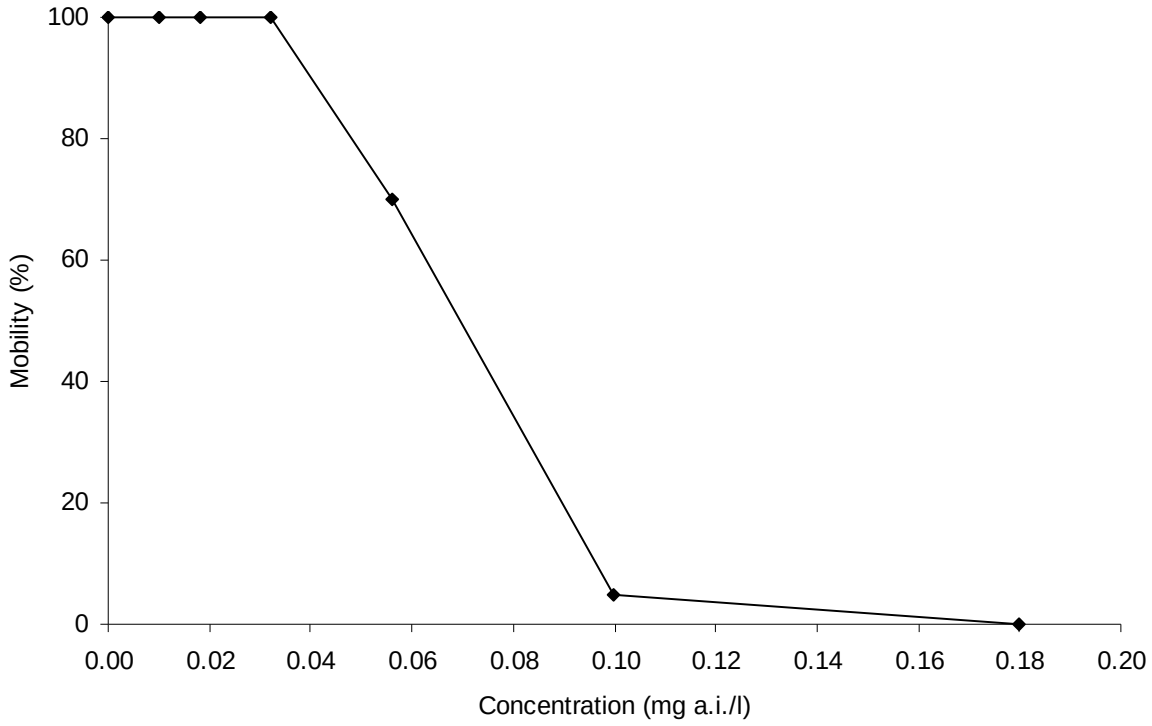

Figure A7.4.1.2- 2: Dose-response relationship after 48 h exposure to TEGO2000.

Table A7.4.1.2-17: Effect data.

	EC_{50}[1]	**95 % CI**	**EC_0[1]**	**EC_{100}[1]**
24 h	0.11	0.1–0.12	0.056	0.18
48 h	0.06	0.06–0.07	0.032	0.18

1) nominal concentrations (mg a.i./l)

Table A7.4.1.2-18: Validity criteria for acute *Daphnia* immobilisation test according to OECD Guideline 202.

	Fulfilled	**Not fulfilled**
Immobilisation of control animals < 10%	☑	
Control animals not staying at the surface	☑	
Concentration of dissolved oxygen in all test vessels > 3 mg/l	☑	
Concentration of test substance ≥ 80% of initial concentration during test	n.a. (not measured)	

Section A7.4.1.2 **Acute toxicity to invertebrates**
Annex Point IIA 7.2

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Reference

Reference	**A7.4.1.2/03:** [redacted] (2007): *Daphnia magna*, acute immobilisation Test (OECD 202) semi-static exposure. Effect of Ampholyt 20 on the immobilization of *Daphnia magna*. Report no. EBR-013/4-20, Fraunhofer-Institute for Molecular Biology and Applied Ecology (IME), Schmallenberg, Germany, July 19, 2007 (unpublished). **A7.4.1.2/04:** [redacted] (2008): Amendment No. 1 to study report *Daphnia magna*, acute immobilisation test (OECD 202) semi-static exposure. Effect of Ampholyt 20 on the immobilization of *Daphnia magna*. Recalculation of effect values based on analytically verified test concentrations. Report no. EBR-013/4-20, Fraunhofer-Institute for Molecular Biology and Applied Ecology (IME), Schmallenberg, Germany, March 20, 2008 (unpublished).
Data protection	Yes
Data owner	Goldschmidt GmbH
Companies with letter of access	No
Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

Guidelines and Quality Assurance

Guideline study	Yes OECD 202 (2004)
GLP	Yes
Deviations	No

Materials and Methods

Test material	As given in Section A2
Lot/Batch number	ES67345616
Specification	As given in Section A2. The active substance as manufactured is obtained by the production process as a “product-by-process”, constituting a 20 % (w/w) aqueous solution of the active matter.
Purity	20 % (w/w) aqueous solution of pure active matter (99 % w/w)
Composition of product	20 % a.i. (aqueous solution, “product by process”)
Further relevant properties	The a.i. is a multi-component substance as specified in Section A2.

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA 7.2

Method of analysis	The concentrations of the test item were assessed by chemical analysis of aliquots taken from fresh and aged test solutions. Fresh medium was sampled at test start and renewal and aged media at renewal and test end. The samples were stored frozen at –18 °C (± 2 °C) until analysis. To assess the concentration of the test item Ampholyt 20, four "lead components" of the mixture were analysed, accounting together for 65.92 % of total active substance or approx. 75 % of its chain length distribution. The concentrations of the single lead compounds were summed up and extrapolated to the total active substance concentration. The test item concentrations were analysed using HPLC-MS/MS. The limit of quantification for each lead compound was 0.1 µg/L. The details of the analytical method are summarised in Section A4.2.
Preparation of TS solution for poorly soluble or volatile test substances	Not applicable
Reference substance	Tests with the reference substance $K_2Cr_2O_7$ are performed in regular intervals, as proposed by OECD 202 (as given in reference A7.4.1.2/03).
Method of analysis for reference substance	Not stated
Testing procedure	
Dilution water	Please refer to Table A7.4.1.2- 19.
Test organisms	Please refer to Table A7.4.1.2- 20.
Test system	Please refer to Table A7.4.1.2-21.
Test conditions	Please refer to Table A7.4.1.2-22.
Duration of the test	Young specimens of *D. magna* of similar age (4–24 h) were exposed to five concentrations of the test item under semi-static conditions for a period of 48 h.
Test parameter	Immobility (after 24 and 48 h).
Sampling	Samples of fresh and aged test solutions were taken from fresh media at test start and renewal and in aged media at renewal (24 h) and test end (48 h). The samples were stored frozen (–18°C, ± 2°C) until analysis.
Monitoring of TS concentration	Ampholyt 20 is a reaction product of alkyl oligoamines and halo acetic acid, obtained as a "product by process" in form of a 20 % (w/w) aqueous solution of the active ingredient. As a result, it represents a mixture of various components. To assess the concentration of the test item Ampholyt 20, four "lead components" of the mixture were analysed (HPLC-MS/MS), accounting together for 65.92 % (w/w) of the active substance [redacted] 35.61 %, [redacted] 4.31 %, [redacted] 10.4 %, [redacted] 15.6 %). The concentrations of the single compounds measured in the test solutions were summed up and extrapolated to the total concentration of the active substance.
Statistics	Probit analysis

Section A7.4.1.2 **Acute toxicity to invertebrates**
Annex Point IIA 7.2

Results

Limit Test	Not performed.
Concentration	–
Number/ percentage of animals showing adverse effects	–
Nature of adverse effects	–
Results test substance	
Initial concentrations of test substance	Nominal: 11.97, 20.35, 34.60, 58.82, and 100.00 µg a.s./L
Actual concentrations of test substance	Detailed data are given in Table A7.4.1.2- 23. In fresh media the measured concentrations of the test item were in the range of 40 % to 137 % of nominal, independent of the nominal concentration. During the 24 h renewal period the test item concentrations decreased to levels of 9 to 61 % of nominal. Recovery rate in aged media was positively correlated with the nominal concentration. For the evaluation of the effect concentrations the time weighted means of the measured concentrations were used. The time weighted mean measured concentrations at the respective treatments levels were calculated to be: 3.29, 6.45, 10.87, 26.98, and 74.29 µg a.s./L (27.5 %, 31.7 %, 31.4 %, 45.9, and 74.3 % of nominal).
Effect data (Immobilisation)	Please refer to Table A7.4.1.2-24.
Concentration/ response curve	Please refer to Figure A7.4.1.2- 3.
Other effects	No
Results of controls	
Test with reference substance	Control: 0 % immobilisation 0.40 mg/L 5 % immobilisation 0.60 mg/L 10 % immobilisation 0.90 mg/L 70 % immobilisation 1.35 mg/L 85 % immobilisation 2.00 mg/L 100 % immobilisation
Concentrations	0.40, 0.60, 0.90, 1.35, 2.00 mg $K_2Cr_2O_7$/L
Results	EC_{50} = 0.83 mg/L (95% CL: 0.73–0.94)

Applicant's Summary and conclusion

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA 7.2

Materials and methods	The influence of Ampholyt 20 on immobilisation of *Daphnia magna* was investigated. For this, the daphnids were placed in water containing the test item in nominal concentrations of 59.87, 101.77, 173.01, 294.10, and 500.00 µg test item per litre, representing 11.97, 20.35, 34.60, 58.82, and 100.00 µg active substance per litre. The test was conducted under semi-static conditions with renewal of test media after 24 h. Effects on immobilisation were determined after 24 and 48 hours. Test substance concentrations were determined by LC-MS/MS. Total active substance concentrations were extrapolated from the mean measured concentrations of four representative lead components.
Results and discussion	Actual test concentrations (time weighted means) were determined to be 3.29, 6.45, 10.87, 26.98, and 74.29 µg a.s./L (27.5 %, 31.7 %, 31.4 %, 45.9, and 74.3 % of nominal). No significantly increased mortality (NOEC) was detected when compared to the control up to mean measured concentrations of 10.87 µg/L. The EC_{50} was estimated at 33.30 µg a.s./L (measured). Thus, the recalculated effect values for immobilization at test end (based on time-weighted mean measured concentrations) are NOEC = 10.87 µg a.s./L LOEC = 26.98 µg a.s./L EC_{10} (95% CL) = 15.86 µg a.s./L (11.21–22.45) EC_{20} (95% CL) = 20.48 µg a.s./L (15.36–27.30) EC_{50} (95% CL) = 33.30 µg a.s./L (26.05–42.57)
EC_0	10.87 µg a.s./L (mean measured concentration)
EC_{50}	33.30 µg a.s./L (mean measured concentration)
EC_{100}	n.d.
Conclusion	
Reliability	1
Deficiencies	None

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	15th January 2013
Materials and Methods	Adopt applicants version.
Results and discussion	Adopt applicants version.
Conclusion	Adopt applicants version.
Reliability	1
Acceptability	Acceptable.
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.4.1.2- 19: Dilution water.

Criteria	Details
Source	Purified drinking water was used as holding- and dilution water. The purification included filtration with activated charcoal, passage through a limestone column, and aeration. To avoid copper contamination, plastic water pipes are used for the testing equipment. The following water chemistry data are recorded regularly in the testing facility and are reported: pH, conductivity, dissolved oxygen content, content of nitrate, nitrite, ammonium (NH_4^+), phosphate, calcium, magnesium, total hardness, alkalinity, DOC content, content of metals (copper, iron, manganese and zinc).
Alkalinity	Not reported
Hardness	0.7–0.8 mmol/l (Ca-hardness 0.4–0.5, Mg-hardness 0.2–0.4)
pH	Not reported
Oxygen content	Not reported
Conductance	Not reported
Holding water different from dilution water	No

Table A7.4.1.2- 20: Test organisms.

Criteria	Details
Species/strain	*Daphnia magna*
Source	*Daphnia magna* (clone V); German Federal Environment Agency, Institut für Wasser-, Boden- und Lufthygiene. Specimens used in the test were bred in the laboratory of the Fraunhofer IME.
Age	4–24 h
Breeding method	Adult *Daphnia*, at least 3 weeks old, were separated from the stock population by sieving. Batches of 30 to 50 animals are held at room temperature in ca. 1.8 L dilution water for one week. During this week the daphnids are fed daily with an algal suspension (*Desmodesmus subspicatus*) and LiquizellR (HOBBY). Algae growing in the log-phase were centrifuged and the pellet was re-suspended in a few mL of medium. 30 mL of this suspension was given to 1 L *Daphnia* medium. The water was changed once per week. Newborn *Daphnia* were separated by sieving, the first generation was discarded.
Kind of food	Algal suspension (*Desmodesmus subspicatus*) and LiquizellR (HOBBY)
Amount of food	Not stated
Feeding frequency	Daily
Pre-treatment	Not reported
Feeding of animals during test	No

Table A7.4.1.2-21: Test system.

Criteria	Details
Renewal of test solution	Yes, the test organisms were transferred in new test vessels with freshly prepared test solutions after 24 hours.
Volume of test vessels	50 ml
Volume/animal	50 ml / 5 animals
Number of animals/vessel	5
Number of vessels/ concentration	4
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.2-22: Test conditions.

Criteria	Details
Test temperature	With temperatures of 20.2–20.4 °C throughout the test the permitted range of 18–22 °C (with a variation of less than 2 °C) was maintained.
Dissolved oxygen	7.5–8.5 mg/L
pH	7.6–8.4 at all treatment levels
Adjustment of pH	No
Aeration of dilution water	No
Quality/intensity of irradiation	The light intensity was measured using an illuminance meter (MINOLTA) with a photometric sensor in Lux: 534–571 lx (corresponding to 9.5–10.2 µE/m²/s)
Photoperiod	16:8 h [light:dark]

Table A7.4.1.2- 23: Measured concentrations of the four lead compounds (sum) in fresh and aged test media and time weighted mean, extrapolated to total active substance (a.s.); lead compounds represent 65.92 % of total a.s.

Nominal conc. of lead compounds (µg/L)	Day 0		Day 1		Day 1		Day 2		Extrapolated time weighted mean (total a.s.)	
	Fresh (µg/L)	% nom.	aged (µg/L)	% nom.	aged (µg/L)	% nom.	aged (µg/L)	% nom.	(µg a.s./L)	% nom.
2.17	3.14	39.8	0.68	8.7	5.65	71.6	1.05	13.3	3.29	27.51
4.25	5.91	44.0	1.98	14.8	8.46	63.0	2.52	18.8	6.45	31.67
7.17	9.16	40.2	3.12	13.7	12.86	56.4	5.59	24.5	10.87	31.42
17.79	33.19	85.6	9.13	23.6	28.02	72.3	9.24	23.8	26.98	45.87
48.97	51.16	77.6	23.91	36.3	90.48	137.3	40.42	61.3	74.29	74.29

Table A7.4.1.2-24: Immobilisation after 24 h and 48 h; a.s. = active substance; concentrations given as mean measured concentrations.

Replicate	Time	Control	3.29 µg a.s./L	6.45 µg a.s./L	10.87 µg a.s./L	26.98 µg a.s./L	74.29 µg a.s./L
	24 h						
1		0	0	20	0	0	20
2		0	0	0	0	0	20
3		0	0	0	0	0	100
4		0	0	0	0	0	20
	48 h						
1		0	0	20	0	20	100
2		0	0	0	0	40	100
3		0	0	0	0	20	100
4		0	0	0	0	0	100

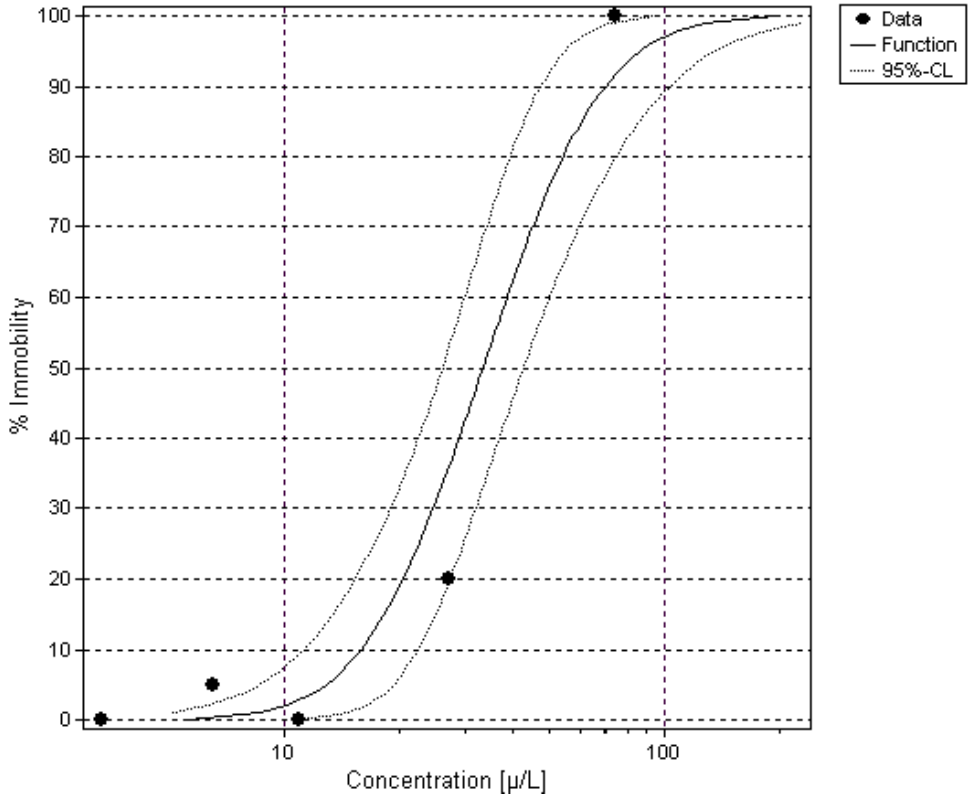

Figure A7.4.1.2- 3: Concentration-effect curve showing the influence of the test item on mobility of the introduced *Daphnia magna* as observed after 48 h.

Table A7.4.1.2-25: Validity criteria for acute *Daphnia* immobilisation test according to OECD Guideline 202.

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

*) The results were therefore evaluated based on measured concentrations

Section A7.4.1.3 **Growth inhibition test on algae**
Annex Point IIA 7.3

Official use only

Reference

Reference	**A7.4.1.3/01:** [redacted] (2002) Ampholyt 20/100 – determination of the growth inhibition of the green algae *Desmodesmus subspicatus*. Infracor GmbH, Marl, Germany, Report no. AW 488, October 01, 2002 (unpublished).
Data protection	Yes
Data owner	Goldschmidt GmbH
Companies with letter of access	No
Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

Guidelines and Quality Assurance

Guideline study	Yes EC C.3 (92/69/EEC) OECD 201 (1984)
GLP	Yes
Deviations	No

Materials and Methods

Test material	Ampholyt 20/100 as given in Section A2.
Lot/Batch number	ES62403356
Specification	As given in Section A2. The active substance as manufactured is obtained by the production process as a “product-by-process”, constituting a 20% (w/w) aqueous solution of the active matter. For the purpose of testing physicochemical properties, the material has exceptionally been lyophilised in order to obtain “pure” active substance, termed “Ampholyt 20/100”. Since the a.s. as manufactured consists of 20% active matter and 80% water, the question if lyophilised “Ampholyt 20/100” or the 20% product is subjected to ecotoxicity testing is considered to be of limited relevance for the reliability of the results.
Purity	99.4%
Composition of product	Not applicable
Further relevant properties	The test material is a multi-component substance as specified in Section A2. A specific method of analysis was not available at the time of test performance. Therefore, TOC analysis was employed to verify concentrations.

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA 7.3

Method of analysis	TOC analysis
Preparation of TS solution for poorly soluble or volatile test substances	Not applicable
Reference substance	No
Method of analysis for reference substance	Not applicable
Testing procedure	
Culture medium	As prescribed by guidelines EC C.3 (92/69/EEC) and OECD 201 (1984).
Test organisms	*Desmodesmus subspicatus*, as described in detail in Table A7.4.1.3- 2.
Test system	Please refer to Table A7.4.1.3- 3.
Test conditions	Details are presented in Table A7.4.1.3- 4.
Duration of the test	72 h
Test parameter	Inhibition of cell multiplication
Sampling	Every 24 h
Monitoring of TS concentration	Monitoring of the test substance in the test medium was not possible since the test concentrations were below the LOQ of the analytical method available at the time of test performance. Therefore, compliance of actual with nominal concentrations was assessed by (i) TOC analysis of the stock solutions (1000 mg/l) and (ii) an additional stability control at a concentration of 2.0 mg/l. Samples for analysis were drawn at 0 h and 72 h.
Statistics	EC_{50}: probit analysis NOEC: Student's t-test

Results

Limit Test	Not performed
Concentration	
Number/ percentage of animals showing adverse effects	
Results test substance	
Initial concentrations of test substance	0.003, 0.005, 0.009, 0.017, 0.030, 0.055, 0.1, 1.0, 2.0 mg/l

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA 7.3

Actual concentrations of test substance	TOC values of the stock solution and an intermediate dilution corresponded to nominal. In the stability control, TS concentrations were maintained within 80% of nominal:

Nominal	**0 h**	**72 h**
Measured	2.06	1.77
% deviation	3	–14

Growth curves	Graphical figures of the growth curves are provided in the original study.
Concentration-response curve	A graph is presented in Figure A7.4.1.3- 1.
Cell concentration data	Please refer to Table A7.4.1.3- 5.
Effect data (cell multiplication inhibition)	E_bC_{50} (72 h) = 0.03 mg/l (95% CI = 0.02–0.03) E_rC_{50} (72 h) = 0.05 mg/l (95% CI = 0.05–0.08) NOE_rC (72 h) = 0.009 mg/l
Other observed effects	In some samples, the pH increased over time. However, since growth rates were not affected, this was considered as an insignificant effect.
Results of controls	See Table A7.4.1.3- 5.
Test with reference substance	Not performed
Concentrations	
Results	

Applicant's Summary and conclusion

Materials and methods	The inhibitory effect of Ampholyt 20/100 on the growth of green algae was tested using *Desmodesmus subspicatus*, according to OECD guideline 201 and EC method C.3. Maintenance of nominal test concentrations was monitored by a parallel stability control as described under 0 oben. Accordingly, it can be safely assumed that actual test concentrations were within 80% of nominal. No deviations from the method prescribed by the guideline were reported.
Results and discussion	The test substance is not known to exhibit any properties that could have affected the outcome of the test.
NOE_rC	0.009 mg/l
E_rC_{50}	0.05 mg/l
E_bC_{50}	0.03 mg/l
Conclusion	All validity criteria were fulfilled (see Table A7.4.1.3- 6). Thus, the study is considered to be valid without restrictions.
Reliability	1
Deficiencies	None

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	
Materials and Methods	Adopt applicants version.
Results and discussion	Nominal values given, not measured due to detection limits at the time.
Conclusion	Study considered valid but with restrictions.
Reliability	3
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.4.1.3- 1: Preparation of TS solution for poorly soluble or volatile test substances.

Criteria	Details
Dispersion	No
Vehicle	No
Concentration of vehicle	Not applicable
Vehicle control performed	Not applicable
Other procedures	None

Table A7.4.1.3- 2: Test organisms.

Criteria	Details
Species	*Desmodesmus subspicatus*
Strain	CHODAT (86.61 SAG)
Source	Albrecht-von-Haller-Institut for plant science, University of Göttingen, Germany
Laboratory culture	Yes
Method of cultivation	Not reported
Pre-treatment	Pre-culture initiated 3 days prior to start of the test
Initial cell concentration	20 000 ml^{-1}

Table A7.4.1.3- 3: Test system.

Criteria	Details
Volume of culture flask	Not reported
Culturing apparatus	Rotary shaker under light benches
Light quality	White-type lamps
Procedure for suspending algae	Shaking
Number of vessels/ concentration	5 (test concentrations) 10 (control)
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.3- 4: Test conditions.

Criteria	Details
Test temperature	23.46°C (range: 23.12–23.72)
pH	Start: 7.4–7.8 End: 7.6–9.8
Aeration of dilution water	Yes, sterile aeration
Light intensity	6000–10000 lux
Photoperiod	Permanent

Table A7.4.1.3- 5: Cell concentration data.

Test substance concentration, nominal/measured [mg/l]	**Cell concentrations**							
	Measured (× 10^4 ml^{-1})				**Percent of control**			
	0 h	*24 h*	*48 h*	*72 h*	*0 h*	*24 h*	*48 h*	*72 h*
Control	2	9	50	97	–	–	–	–
0.003	2	9	51	98	100	100	102	101
0.005	2	8	58	91	100	89	116	94
0.009	2	8	42	89	100	89	84	92
0.017	2	7	32	81	100	78	64	84
0.030	2	8	23	56	100	89	46	58
0.055	2	5	13	25	100	56	26	26
0.1	2	1	0	1	100	11	0	1
1.0	2	1	0	0	100	11	0	0
2.0	2	2	0	0	100	22	0	0
Temperature [°C]	23.46°C (range: 23.12–23.72)							
pH	Start: 7.4–7.8 End: 7.6–9.8							

Table A7.4.1.3- 6: Validity criteria for algal growth inhibition test according to OECD Guideline 201.

	Fulfilled	**Not fulfilled**
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	☑	
Concentration of test substance ≥80% of initial concentration during test	☑	
Criteria for poorly soluble test substances	Not applicable	

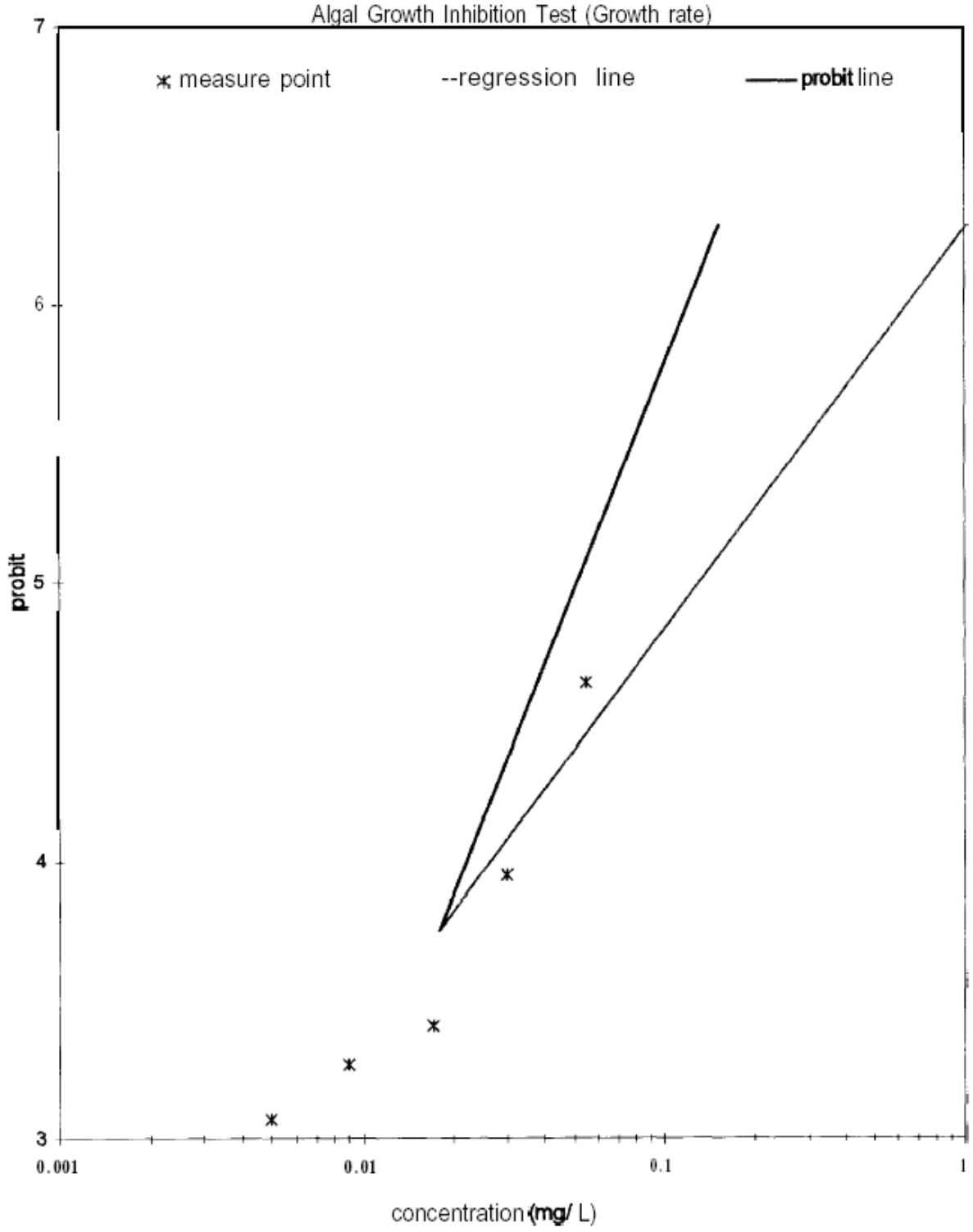

Figure A7.4.1.3- 1: Dose-response relationship (growth rate) of the green alga *Desmodesmus subspicatus* by Ampholyt 20/100.

Section A7.4.1.3 **Growth inhibition test on algae**
Annex Point IIA 7.3

Official use only

Reference

Reference	**A7.4.1.3/02**: [redacted] (1995): Effect of TEGO 2000 on the growth of the green alga *Selenastrum capricornutum*. TNO Institute of Environmental Sciences, Delft, The Netherlands, unpublished report no. TNO-MW-R 95/001, January 31, 1995.
Data protection	Yes
Data owner	Goldschmidt GmbH
Companies with letter of access	No
Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

Guidelines and Quality Assurance

Guideline study	Yes OECD 201 (1984)
GLP	Yes
Deviations	No chemical analysis was carried out. The detection limit of the analytical method available to that time was higher (0.4 mg a.i./l) than the concentrations used in this test. Only duplicate test concentrations were prepared, instead of triplicates demanded by OECD 201 (1984), without justification.

Materials and Methods

Test material	TEGO 2000 (synonym/trade name for Ampholyt 20), please refer to Section A2. 20% a.i. (aqueous solution, “product by process”)
Lot/Batch number	490486
Specification	Ampholyt 20 as given in Section A2. The active substance as manufactured is obtained by the production process as a “product-by-process”, constituting a 20% (w/w) aqueous solution of the active matter.
Purity	The active substance in itself is considered as pure (≥ 99%).
Composition of product	20% a.i. (aqueous solution, “product by process”)
Further relevant properties	The a.i. is a multi-component substance as specified in Section A2. A specific method of analysis was not available at the time of test performance.

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA 7.3

Method of analysis	The actual concentrations of the a.i. of the test substance in the test solutions were not determined by chemical analysis (non-availability of a validated method). TEGO 2000 (20% a.i.) was diluted to the test concentrations (see below). The final test was performed using seven concentrations (plus control) of active ingredient: 0, 0.002, 0.006, 0.011, 0.020, 0.037, 0.065, and 0.204 mg a.i./l.
Preparation of TS solution for poorly soluble or volatile test substances	Not required
Reference substance	The test systems used are checked regularly by testing both of the reference substances $K_2Cr_2O_7$ and 3,5-dichlorophenol and participation in international ring tests. The EC values obtained with the reference substances are constant and similar to the results of the ring test. Values or more information are not given in the report.
Method of analysis for reference substance	Not stated.
Testing procedure	
Culture medium	The composition of the algal medium is given in Table A7.4.1.3-8.
Test organisms	Freshwater green alga *Selenastrum capricornutum*, see Table A7.4.1.3-7
Test system	See Table A7.4.1.3-8
Test conditions	See Table A7.4.1.3-9
Duration of the test	72 h
Test parameter	Growth inhibition
Sampling	After 3 days of incubation one sample was taken from each flask, and the number of particles per ml in the samples was analysed with the aid of a Coulter Counter model TAII.
Monitoring of TS concentration	Not performed due to the non-availability of a suitable analytical method.
Statistics	EC_{50} by logistic regression. NOEC by visual comparison of growth rates.

Results

Limit Test	Not performed
Concentration	
Number/ percentage of animals showing adverse effects	
Results test substance	
Initial concentrations of test substance	Nominal concentrations: 0.002, 0.006, 0.011, 0.020, 0.037, 0.065 and 0.204 mg active ingredient (a.i.)/L.

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA 7.3

Actual concentrations of test substance	Test concentrations could not be verified due to non-availability of a suitable analytical method.		
Growth curves	Please refer to the study report, Figure 1.		
Concentration-response curve	Please refer to the study report, Figure 2.		
Cell concentration data	See Table A7.4.1.3-10.		
Effect data (cell multiplication inhibition)	E_rC_{10}	0.0021 mg (a.i.) /l	
	E_rC_{50}	0.0077 mg (a.i.) /l	(95% CI = 0.0056–0.011)
	E_rC_{90}	0.0280 mg (a.i.) /l	
	E_bC_{10}	0.0030 mg (a.i.) /l	(95% CI = 0.002–0.006)
	E_bC_{50}	0.0160 mg (a.i.) /l	(95% CI = 0.006–0.020)
	E_bC_{90}	0.0250 mg (a.i.) /l	(95% CI = 0.011–0.037)
Other observed effects	Cell deformations were found at test substance treatment concentrations of 0.011 mg (a.i.) /l and higher.		
Results of controls	See Table A7.4.1.3-10.		
Test with reference substance	Not reported		
Concentrations	n.a.		
Results	n.a.		

Applicant's Summary and conclusion

Materials and methods	The toxicity of TEGO 2000 to the freshwater green alga *Selenastrum capricornutum* was determined in a growth inhibition test according to the OECD Guideline No. 201. The concentrations of TEGO 2000 tested were 0.002, 0.006, 0.011, 0.020, 0.037, 0.065 and 0.204 mg active ingredient (a.i.)/l. The algal growth was determined by electronic particle counting. The effect values were calculated using a parametric model (logistic regression) assuming an error constant per measurement.
Results and discussion	The test substance is not known to exhibit any properties that could have affected the outcome of the test.
NOE_rC	0.002 mg a.i./l
E_rC_{50}	0.0077 mg a.i./l
E_bC_{50}	0.0160 mg a.i./l
Conclusion	Due to the deficiencies given in 5.3.2 the reliability is lowered to 3.
Reliability	3

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA 7.3

Deficiencies	No chemical analysis was carried out (non-availability of an analytical method to that time).Only duplicate test concentrations were prepared, instead of triplicates demanded by OECD 201 (1984), without justification. The test conditions are only poorly documented. In view of these deficiencies, the study must be considered as of limited validity.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	15th January 2013
Materials and Methods	No chemical analysis was carried out (not available at that time).Only duplicate test concentrations were prepared, instead of triplicates demanded by OECD 201 (1984), without justification.
Results and discussion	The test conditions are poorly documented.
Conclusion	In view of these deficiencies, the study is unreliable.
Reliability	3
Acceptability	No
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.4.1.3- 7: Test organisms.

Criteria	Details
Species	*Selenastrum capricornutum* (Chlorococcales)
Strain	ATCC22662
Source	American Type Culture Collection, c/o Sales Department 12301 Oarklawn Drive, Rockville, Maryland 20852, USA.
Laboratory culture	Yes
Method of cultivation	Not reported
Pre-treatment	Pre-culture according to OECD 201
Initial cell concentration	1.0×10^4 cells per ml

Table A7.4.1.3-8: Test system.

Criteria	Details	
Volume of culture flask	200 ml	
Composition of algal medium	NH_4C1	15 mg/l
	$MgC1_2 \times 6H_2O$	12 mg/l
	$CaCl_2 \times 2 H_2O$	18 mg/l
	$MgSO_4 \times 7 H_2O$	15 mg/l
	KH_2PO_4	1.6 mg/l
	Fe-citrate $\times 3 H_2O$	80 µg/l
	$Na_2EDTA \times 2 H_2O$	100 µg/l
	H_3BO_3	185 µg/l
	$MnCl_2 \times 4 H_2O$	415 µg/l
	$ZnCl_2$	3 µg/l
	$CoCl_2 \times 6 H_2O$	1.5 µg/l
	$CuCl_2 \times 2 H_2O$	0.01 µg
	$Na_2MoO_4 \times 2 H_2O$	7 µg/l
	$NaHCO_3$	150 mg/l (to assure the buffer capacity of the medium)
Culturing apparatus	Flasks placed on a Gallenkamp orbital shaker (100 rpm)	
Light quality	Fluorescent lamp 60–120 µmol $\times s^{-1} \times m^{-2}$, measured with a Bottemanne Weather Instruments Photosynthetic Radiometer RA200 Q.	
Procedure for suspending algae	All flasks were incubated at 23 ± 1°C and shaken (approximately 100 rpm) in a Gallenkamp orbital shaker.	
Number of vessels/ concentration	2 vessels per concentration, 4 controls	
Test performed in closed vessels due to significant volatility of TS	No	

Table A7.4.1.3-9: Test conditions.

Criteria	Details
Test temperature	23 ± 1 °C
pH	The pH of the algal medium (measured at the start (without algae) and after 71.5 h) containing different test substance concentrations, in the presence and absence of algal cells, was found to be stable during the test (pH 8.3–8.1). The highest pH value reached in the test medium was pH 8.3, which is well below the limit given in the guideline.
Aeration of dilution water	Not reported
Light intensity	60–120 µmo1 × s^{-l} × m^{-2} (measured with a Bottemanne Weather Instruments Photosynthetic Radiometer RA200 Q)
Photoperiod	Although not explicitly stated, continuous lighting may be assumed

Table A7.4.1.3-10: Cell concentration data.

Test substance concentration, nominal [mg/l]	**Mean cell counts**							
	Measured (× 10^4 $ml^{-1,}$ corrected for background)				**Percent of control**			
	0 h	*24 h*	*48 h*	*72 h*	*0 h*	*24 h*	*48 h*	*72 h*
0	1.2	3.6	8.35	18.7	100.00	100.00	100.00	100.00
0.002	1.2	3.5	9.2	18.4	100.00	97.22	110.18	98.40
0.006	1.2	3.2	8	13.7	100.00	88.89	95.81	73.26
0.011	1.2	2.3	4.9	7.6	100.00	63.89	58.68	40.64
0.02	1.2	2.4	5.3	7.6	100.00	66.67	63.47	40.64
0.037	1.2	0.7	0.8	0.8	100.00	19.44	9.58	4.28
0.065	1.2	0.5	0.4	0.6	100.00	13.89	4.79	3.21
0.204	1.1	0.4	0.3	0.3	91.67	11.11	3.59	1.60

Table A7.4.1.3- 11: Validity criteria for algal growth inhibition test according to OECD Guideline 201.

	Fulfilled	**Not fulfilled**
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	☑	
Concentration of test substance ≥ 80% of initial concentration during test	n.a. (not measured)	
Criteria for poorly soluble test substances	Not applicable	
Limited increase in the pH of the test medium (1 pH unit)	☑	

Section A7.4.1.3 **Growth inhibition test on algae**
Annex Point IIA 7.3

Official use only

Reference

Reference	**A7.4.1.3/03:** [redacted] (2007): Alga, growth inhibition test. Effect of Ampholyt 20 on the Growth of *Pseudokirchneriella subcapitata*, static conditions. Fraunhofer-Institute for Molecular Biology and Applied Ecology (IME), Schmallenberg, Germany, June 26, 2007. **A7.4.1.3/04:** [redacted] (2008): Amendment to Study Report: Alga, growth inhibition test. Effect of Ampholyt 20 on the Growth of *Pseudokirchneriella subcapitata*, static conditions. Fraunhofer-Institute for Molecular Biology and Applied Ecology (IME), Schmallenberg, Germany, November 12, 2008.
Data protection	Yes
Data owner	Goldschmidt GmbH
Companies with letter of access	No
Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

Guidelines and Quality Assurance

Guideline study	Yes OECD 201 (2006)
GLP	Yes
Deviations	The validation of the analytical method had not been finalised at termination of the algal growth test. After completion of the validation of the analytical method, this method will be recorded in detail and added to the study report and this study summary.

Materials and Methods

Test material	Ampholyt 20 as given in Section A2.
Lot/Batch number	ES67345616
Specification	Ampholyt 20 as given in Section A2. The active substance as manufactured is obtained by the production process as a “product-by-process”, constituting a 20% (w/w) aqueous solution of the active matter.
Purity	99 % w/w
Composition of product	Not applicable

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA 7.3

Further relevant properties	The test material is a multi-component substance as specified in Section A2. Thus, analytical verification of test substance concentrations employed a lead substance concept, focussing on the C_{12}-alkyl compounds only.
Method of analysis	The validation of the analytical method had not been finalised at termination of the algal growth test. After completion of the validation the method the concentrations of the test item were assessed by chemical analysis in November 2007 (reference A7.4.1.3/04)
Preparation of TS solution for poorly soluble or volatile test substances	Not applicable due to the high solubility of the test substance.
Reference substance	None
Method of analysis for reference substance	Not applicable
Testing procedure	
Culture medium	Without deviation to the Guideline OECD 201. The composition of the algal medium is given in Table A7.4.1.3-13.
Test organisms	*Pseudokirchneriella subcapitata*, Chlorophycea, Chlorophyta, as described in Table A7.4.1.3- 12.
Test system	See Table A7.4.1.3-13
Test conditions	See Table A7.4.1.3-14
Duration of the test	72 h
Test parameter	Growth inhibition
Sampling	At test start samples of the highest, medium and lowest test concentrations and the control were taken from the test solution preparations just before distributing it to the replicates. During the test whole test vessels were used as sample. The sampling scheme is given in Table A7.4.1.3- 15.
Monitoring of TS concentration	The concentrations of the test item at day 0. 1, 2 and 3 (end of test) were assessed by chemical analysis of three test concentrations below, equal and above the nominal NOEC. To assess the influence of algae on the test item concentrations during the test, one supplementary replicate without algae was prepared and analysed at the medium and highest test item concentration, respectively.
Statistics	EC_{10} and EC_{50} values (together with 95 % confidence intervals) by using Probit-analysis (computer program ToxRat®) NOEC by Williams Multiple Sequential t-test.

Results

Limit Test	Not performed

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA 7.3

Concentration	
Number/ percentage of animals showing adverse effects	
Results test substance	
Initial concentrations of test substance	Nominal concentrations: 1.25, 2.50, 5.00, 10.0 and 20.0 µg/L active matter of Ampholyt 20.
Actual concentrations of test substance	At test start the measured concentrations of the test item in the treatment of 5 and 20 µg a.s./L were 126 % and 79.9 % of nominal. During the test the test item levels decreased to 20.1 and 19.9 % of nominal, respectively, at test end. The analytical data obtained from the 10 µg/L treatment had to be excluded from the evaluation due to obvious measurement errors (most likely shortcomings in sample preparation). It could be shown that the algae did not influence the test item concentrations over the exposure period.
Growth curves	The growth curves are given in Figure A7.4.1.3- 2
Concentration-response curve	The concentration-effect curves showing the influence of the nominal concentration of the test item on % inhibition of growth rate, yield (cell number increase) or decrease of biomass integral as observed after 72 h are given in the original study.
Cell concentration data	Please refer to Table A7.4.1.3-16 below.
Effect data (cell multiplication inhibition)	For percent inhibition of growth rate see Table A7.4.1.3- 17. Effective concentrations (after 72 hours): Growth rate (r) EC_{50} > 20.0 µg/L EC_{10} = 17.8 µg/L (95 % CL = 16.9–18.3) NOEC = 10.0 µg/L Yield (y) EC_{50} = 18.7 µg/L (95 % CL = 18.6–18.8) EC_{10} = 14.5 µg/L (95 % CL = 14.1–14.8) NOEC = 10.0 µg/L Biomass integral(B) EC_{50} = 18.7 µg/L (95 % CL n.d) EC_{10} = 14.5 µg/L (95 % CL n.d) NOEC = 10.0 µg/L (n.d.: not determined, for mathematical reasons)
Other observed effects	None
Results of controls	
Test with reference substance	No test with reference substance was performed
Concentrations	n.a.
Results	n.a.

Applicant's Summary and conclusion

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA 7.3

Materials and methods	The toxicity of the a.i. of the test item Ampholyt 20 on the growth of the green alga *Pseudokirchneriella subcapitata* was determined according to OECD guideline 201. The test was designed to determine the NOEC for the measured parameters. The algae were exposed to nominal concentrations of 1.25, 2.50, 5.0, 10.0 and 20 µg active matter of Ampholyt 20/L under static conditions for 72 hours. For the determination of algal growth, three replicates for each concentration and six replicates for controls (test medium only) were used.
Results and discussion	There was a concentration dependent inhibition of algal growth at exposure levels > 10.0 µg/L (nominal). At the highest test item concentration of 20 µg/L algal growth rate, yield and biomass were inhibited to 18.1 %, 62.8 % and 63.9 %, respectively. The 72 h EC_{50} values for both yield and biomass were calculated to be 18.7 µg/L. The 72 h EC_{50} value for growth rate was > 20.0 µg/L. The NOEC values for all three parameters was 10.0 µg test item/L.
NOE_rC	10.0 µg a.i./l (nominal)
E_rC_{50}	> 20.0 µg a.i./l (nominal)
E_bC_{50}	18.7 µg a.i./l (nominal)
Conclusion	Most validity criteria were fulfilled (see Table A7.4.1.3- 18). However, due to measurement errors only two test concentrations could be monitored analytically, showing more than 20 % decrease of the active substance during the test period. According to OECD 201 the concentrations of all treatment levels should be analytically verified if the test item is not stable during the test. Thus, the study cannot be considered valid and a repeat study was performed (see reference A7.4.1.3/05, summarised below).
Reliability	4
Deficiencies	Test item concentrations: The nominal concentrations were used for a preliminary evaluation, since the validation of the analytical method was not finalised at termination of the algal growth test. After completion of the validation the analytical measurements revealed a decrease of the concentration of test substance of more than 80 % of initial concentration during test. The test is therefore considered to be invalid.

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	16th January 2013
Materials and Methods	Adopt applicants version.
Results and discussion	Adopt applicants version.
Conclusion	Adopt applicants version.
Reliability	4
Acceptability	No
Remarks	Study repeated A7.4.1.3/05.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.4.1.3- 12: Test organisms.

Criteria	Details
Species	*Pseudokirchneriella subcapitata*, Chlorophycea, Chlorophyta.
Strain	SAG 61.81
Source	SAG, Culture Collection of Algae at Pflanzenphysiologisches Institut of the University at Göttingen, Albrecht von Haller Institut, Untere Karspüle 2, 37073 Göttingen, Germany. Catalog No 61.81.
Laboratory culture	Yes
Method of cultivation	The stock cultures were maintained fulfilling the criteria of the OECD guidelines (1).
Pre-treatment	Three days prior to testing a pre-culture was established in OECD growth medium to obtain exponentially growing algae for the test.
Initial cell concentration	10 000 cells/mL

Table A7.4.1.3-13: Test system.

Criteria	Details
Volume of culture flask	100 ml
Composition of algal medium	Algal medium according to OECD 201: $NaHCO_3$ 50 NH_4Cl 15 K_2HPO_4 1.6 $MgSO_4 \cdot 7\ H_2O$ 15 $MgCl_2 \cdot 6\ H_2O$ 12 $CaCl_2 \cdot 2\ H_2O$ 18 $FeCl_3 \cdot 6\ H_2O$ 0.064 H3BO3 0.185 $MnCl_2 \cdot 4\ H_2O$ 0.415 $ZnCl_2$ 0.003 $CoCl_2 \cdot 6\ H_2O$ 0.0015 $CuCl_2 \cdot 2\ H_2O$ 0.00001 $Na_2MoO_4 \cdot 2\ H_2O$ 0.007 $Na_2EDTA \cdot 2\ H_2O$ 0.1 pH, at test start approx. 8.0
Culturing apparatus	250 mL conical glass flasks covered with silicone-sponge caps placed on a laboratory shaker at 100 rpm (Incubation Shaker Multitron ®, INFORS, Switzerland)
Light quality	Light intensity was between 6720 and 6880 lux (equivalent to approximately 91-93 µE m^{-2} s^{-1}). The light intensity was measured daily using an illuminance meter LI-189 (LI-COR, Lincoln, USA with radiation sensor) with a cosine (2π) receptor in lux.
Procedure for suspending algae	(Round)shaking movements on a laboratory shaker at 100 rpm (Incubation Shaker Multitron ®, INFORS, Switzerland). For preparing the test cultures for the growth test, every flask was filled with 100 mL of the respective test medium. 0.870 mL of the pre-culture (cell density 1.149 x 10^6 cells/mL) was added to the test vessels to achieve the initial cell concentration of 10 000 cells/mL.
Number of vessels/ concentration	3 replicates per concentration, 6 controls
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.3-14: Test conditions.

Criteria	Details
Test temperature	22.5–23.0 °C
pH	The pH values were measured in an additionally prepared replicate at test start and directly in the test vessels at the end of the test. During the exposure the incubation temperature was measured once a day in an additionally prepared control vessel, which was continuously incubated.

C [µg/l]	Control	1.25	2.50	5.00	10.0	20.0
Test start	8.04	8.03	8.02	8.05	8.03	8.09
Test end	8.87	8.64	8.49	8.23	8.12	8.09

Aeration of dilution water	Not reported
Light intensity	Light intensity was between 6720 and 6880 lux (equivalent to approximately 91–93 µE m^{-2} s^{-1}). The light intensity was measured daily using an illuminance meter LI-189 (LI-COR, Lincoln, USA with radiation sensor) with a cosine (2π) receptor in lux.
Photoperiod	Continuous lighting

Table A7.4.1.3- 15: Scheme for sampling and treatment of samples:

	Test cultures			**Supplementary test vessels without algae**	
Test duration	Test medium (supernatant of algae cultures)	Algae (centrifuged)	Elution of wall of test vessel	Test medium	Elution of wall of test vessel
0 h	4*	–		–	–
	(1 replicate of highest, medium and lowest treatments, 1 control)				
24 h	3	3	3	–	–
	(1 replicate of highest, medium and lowest treatments)				
48 h	3	3	3	–	–
	(1 replicate of highest, medium and lowest treatments)				
72 h	10	10	10	3	3
	(3 replicates of highest, medium and lowest treatments, 1 control)			1 replicate of highest, medium and lowest treatments	

Table A7.4.1.3-16: Cell number (× 10^4) and yield (= final cell number minus initial cell number of 10 000) dependent on nominal concentrations of the test item and time.

Test substance concentration, nominal [µg/l]	Cell concentrations (mean values) [cells/ml]							
	Measured (× 10^4 ml^{-1})				Percent of control			
	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
Control	1	5.845	33.843	216.167	100	100	100	100
1.25	1	6.167	35.337	281.100	100	106	104	130
2.50	1	6.803	38.127	296.667	100	116	113	137
5.00	1	5.010	35.700	272.767	100	85.7	106	126
10.0	1	3.912	30.837	261.067	100	66.9	91.1	121
20.0	1	3.174	11.333	81.020	100	54.3	33.5	37.5

Table A7.4.1.3- 17: Growth rate (G) and its inhibition relative to control (%I) as computed from the raw data for test intervals selected.

Treatment	0–24 h		0–48 h		0–72 h	
[µg/L]	G	%I	G	%I	G	%I
Control	1.762	0.0	1.760	0.0	1.789	0.0
1.25	1.818	–3.2	1.782	–1.3	1.877	–4.9
2.50	1.917	–8.8	1.820	–3.4	1.896	–6.0
5.00	1.610	8.6	1.787	–1.5	1.869	–4.5
10.0	1.361	22.8	1.713	2.7	1.853	–3.6
20.0	1.155	34.5	1.214	31.0	1.465	18.1

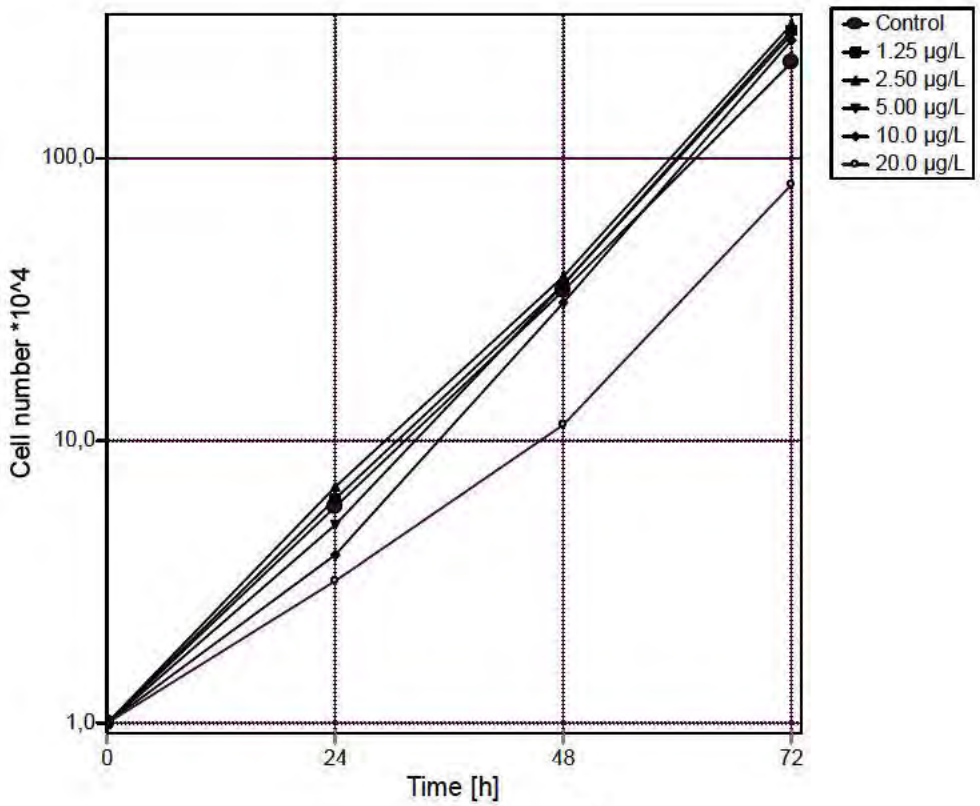

Figure A7.4.1.3- 2: Cell number (× 10^4) of *Pseudokirchneriella subcapitata* dependent on nominal concentrations of the test item (a.i. Ampholyt 20).

Table A7.4.1.3- 18: Validity criteria for algal growth inhibition test according to OECD Guideline 201.

	Fulfilled	Not fulfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	☑	
Concentration of test substance ≥ 80% of initial concentration during test		☑
Criteria for poorly soluble test substances	Not applicable	
Limited increase in the pH of the test medium (1 pH unit)	☑	

Section A7.4.1.3 **Growth inhibition test on algae**
Annex Point IIA 7.3 **– Key study –**

Official use only

Reference

Reference	**A7.4.1.3/05:** [redacted] (2008): Alga, growth inhibition test. Effect of Ampholyt 20 on the Growth of *Pseudokirchneriella subcapitata*, static conditions. Fraunhofer-Institute for Molecular Biology and Applied Ecology (IME), Schmallenberg, Germany, Report No. EBR-13/4-30/1, March 31, 2008 (unpublished).
Data protection	Yes
Data owner	Goldschmidt GmbH
Companies with letter of access	No
Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

Guidelines and Quality Assurance

Guideline study	Yes OECD 201 (2006)
GLP	Yes
Deviations	No

Materials and Methods

Test material	Ampholyt 20 as given in Section A2.
Lot/Batch number	ES67345616
Specification	Ampholyt 20 as given in Section A2. The active substance as manufactured is obtained by the production process as a “product-by-process”, constituting a 20 % (w/w) aqueous solution of the active matter.
Purity	99 % w/w
Composition of product	Not applicable
Further relevant properties	The test material is a multi-component substance as specified in Section A2. Thus, analytical verification of test substance concentrations employed a lead substance concept, focussing on the C_{12}-alkyl compounds which are considered as representative for the complex mixture.

Section A7.4.1.3 **Growth inhibition test on algae**
Annex Point IIA 7.3 **– Key study –**

Method of analysis	The test item concentrations were analysed using HPLC-MS/MS. To assess the concentration of the test item Ampholyt 20, four "lead components" of the mixture were analysed, accounting together for 65.92 % of total active substance. The concentrations of the single lead compounds were summed up and extrapolated to the total active substance concentration. The details are summarised in Section A4.2.
Preparation of TS solution for poorly soluble or volatile test substances	No
Reference substance	None
Method of analysis for reference substance	Not applicable
Testing procedure	
Culture medium	According to OECD guideline 201. The composition of the algal medium is given in Table A7.4.1.3-20.
Test organisms	*Pseudokirchneriella subcapitata*; for details please refer to Table A7.4.1.3- 19.
Test system	Growth inhibition, static conditions; for details please refer to Table A7.4.1.3-20.
Test conditions	Please refer to Table A7.4.1.3- 21 below.
Duration of the test	72 h
Test parameter	Inhibition of cell multiplication
Sampling	Every 24 h
Monitoring of TS concentration	Yes Concentrations of Ampholyt 20 in terms of total active substance were measured daily.
Statistics	The test results were statistically analysed to determine EC_{10} and EC_{50} values and their 95 % confidence intervals using probit-analysis assuming log-normal distribution of the values using the computer program ToxRat®.

Results

Limit Test	No
Concentration	
Number/ percentage of animals showing adverse effects	
Results test substance	
Initial concentrations of test substance	The measured initial concentrations, as determined by LC-MS/MS, are given in Table A7.4.1.3- 23.

Section A7.4.1.3 **Growth inhibition test on algae**
Annex Point IIA 7.3 **– Key study –**

Actual concentrations of test substance	The mean measured concentrations, as determined by LC-MS/MS, are given in Table A7.4.1.3- 23.
Growth curves	A graphical figure is given in Figure A7.4.1.3- 3 below.
Concentration-response curve	Concentration-response curves are presented in Figure A7.4.1.3- 4, Figure A7.4.1.3- 5, and Figure A7.4.1.3- 6 for growth, yield, and biomass integral, respectively.
Cell concentration data	The data are given in Table A7.4.1.3-24 below.
Effect data (cell multiplication inhibition)	The effect data are given in Table A7.4.1.3- 25 below. E_bC_{50} (72 h) = 19.5 µg/l (95% CI = 19.4–19.5) E_rC_{50} (72 h) = 23.7 µg/L (23.2–24.5 µg/L), extrapolated, since the highest test concentration was 12.1 µg a.s./L NOEC (72 h) = 9.55 µg/L
Other observed effects	Due to the decrease of the exposure concentrations during the test period, the mean measured concentrations were used for the evaluation of the effect concentrations (geometric mean of the measured concentrations). By comparison of concentrations measured in test media without algae, it could be shown that the algae did not influence the test item concentration.
Results of controls	See Table A7.4.1.3-24.
Test with reference substance	n.a.
Concentrations	
Results	

Applicant's Summary and conclusion

Materials and methods	In a growth inhibition test with *Pseudokirchneriella subcapitata* the effects of Ampholyt 20 under static conditions for 72 hours (according to OECD guideline 201) were assessed. Due to the decrease of the test substance of more than 20 % over the exposure period, the test results were based on mean measured concentrations (geometric mean).
Results and discussion	The EC_{50} value for growth rate was only slightly higher than the highest test concentration of 21.1 µg a.s./L. It is therefore regarded feasible to use the extrapolated value as endpoint. The NOEC values for growth rate, yield and biomass over 72 h were determined at 9.55 µg a.s./L (mean measured concentration).
NOE_rC	9.55 µg a.s./L
E_rC_{50}	23.7 µg a.s./L (23.2–24.5 µg/L)
E_bC_{50}	19.5 µg a.s./L (19.4–19.5 µg/L)
E_yC_{50}	19.3 µg a.s./L (19.2–19.3 µg/L)
Conclusion	
Reliability	1
Deficiencies	None

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	16th January 2013	
Materials and Methods	Applicant should have tested more concentrations between 9.55 and 21.1 µg a.s./L.	
Results and discussion	Adopt applicants version.	
Conclusion	Adopt applicants version.	
Reliability	2	
Acceptability	Acceptable	
Remarks		
	COMMENTS FROM ...	
Date		
Materials and Methods		
Results and discussion		
Conclusion		
Reliability		
Acceptability		
Remarks		

Table A7.4.1.3- 19: Test organisms.

Criteria	Details
Species	*Pseudokirchneriella subcapitata*, Chlorophycea, Chlorophyta
Strain	SAG 61.81
Source	SAG, Culture Collection of Algae at Pflanzenphysiologisches Institut of the University at Göttingen, Albrecht von Haller Institut, Untere Karspüle 2, 37073 Göttingen, Germany. Catalog No 61.81
Laboratory culture	Yes
Method of cultivation	The stock cultures were maintained fulfilling the criteria of the OECD guideline
Pre-treatment	Three days prior to testing a pre-culture was established in OECD growth medium to obtain exponentially growing algae for the test
Initial cell concentration	10 000 cells/mL

Table A7.4.1.3-20: Test system.

Criteria	Details
Volume of culture flask	100 mL
Composition of algal medium	Algal medium according to OECD 201: $NaHCO_3$ 50 mg/L NH_4Cl 15 mg/L K_2HPO_4 1.6 mg/L $MgSO_4 \cdot 7\ H_2O$ 15 mg/L $MgCl_2 \cdot 6\ H_2O$ 12 mg/L $CaCl_2 \cdot 2\ H_2O$ 18 mg/L $FeCl_3 \cdot 6\ H_2O$ 0.064 mg/L H_3BO_3 0.185 mg/L $MnCl_2 \cdot 4\ H_2O$ 0.415 mg/L $ZnCl_2$ 0.003 mg/L $CoCl_2 \cdot 6\ H_2O$ 0.0015 mg/L $CuCl_2 \cdot 2\ H_2O$ 0.00001 mg/L $Na_2MoO_4 \cdot 2\ H_2O$ 0.007 mg/L $Na_2EDTA \cdot 2\ H_2O$ 0.1 mg/L pH at test start approx. 8.0
Culturing apparatus	250 mL conical glass flasks covered with silicone-sponge caps placed on a laboratory shaker at 100 rpm (Incubation Shaker Multitron ®, INFORS, Switzerland)
Light quality	7000 Lux (95 µE × m^{-2} × s^{-1})
Procedure for suspending algae	(Round)-shaking movements on a laboratory shaker at 100 rpm (Incubation Shaker Multitron ®, INFORS, Switzerland). For preparing the test cultures for the growth test, every flask was filled with 100 mL of the respective test medium. 303 µL of the pre-culture (cell density 3.3 × 10^6 cells/mL) were added to the test vessels to achieve the initial cell concentration of 10 000 cells/mL
Number of vessels/ concentration	3 replicates per concentration, 6 controls
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.3- 21: Test conditions.

Criteria	Details
Test temperature	Please refer to Table A7.4.1.3-24
Composition of algal medium	See Table A7.4.1.3-20 above.
pH	The pH values were measured in an additionally prepared replicate at test start and directly in the test vessels at the end of the test. C [µg/l] Control 1.1 2.2 4.57 9.55 21.1 Test start 7.92 7.94 7.91 7.90 7.93 7.88 Test end 7.88 8.51 8.49 8.50 8.49 8.47
Aeration of dilution water	Not reported
Light intensity	Constant 7320 Lux, measured daily using an illuminance meter LI-189 (LI-COR, Lincoln, USA with radiation sensor) with a cosine (2π) receptor in lux
Photoperiod	Continuously

Table A7.4.1.3- 22: Scheme for sampling and treatment of samples.

	Test cultures			Supplementary test vessels without algae	
Test duration	**Test medium (supernatant of algae cultures)**	**Algae (centrifuged)**	**Elution of wall of test vessel**	**Test medium**	**Elution of test vessel wall**
0 h	4	–		–	–
	(1 replicate of highest, medium and lowest treatments, 1 control)				
24 h	3	3	3	–	–
	(1 replicate of highest, medium and lowest treatments)				
48 h	3	3	3	–	–
	(1 replicate of highest, medium and lowest treatments)				
72 h	10	10	10	3	3
	(3 replicates of highest, medium and lowest treatments, 1 control)			1 replicate of highest, medium and lowest treatments	

Table A7.4.1.3- 23: Measured concentrations of Ampholyt 20 in terms of total active substance during the growth inhibition test with *Pseudokirchneriella subcapitata.*

Nom. conc. (µg a.s./L)	**Day 0**		**Day 1**		**Day 2**		**Day 3**		**Mean measured [1], day 0–3**	
	Fresh (µg/L)	**% nom.**	**Aged (µg/L)**	**% nom.**	**Aged (µg/L)**	**% nom.**	**Aged (µg/L)**	**% nom.**	**(µg/L)**	**% nom.**
Control	< LOQ	–	< LOQ	–	< LOQ	–	< LOQ	–	< LOQ	–
2.5	1.93	77.2	1.00	40.1	1.06	42.4	0.72	28.9	1.10	44.2
5.0	3.98	79.6	1.60	32.1	1.88	37.5	1.96	39.3	2.20	44.1
10.0	7.47	74.7	3.63	36.3	3.78	37.8	4.24	42.4	4.57	45.7
20.0	18.0	90.0	9.42	47.1	7.08	35.4	6.94	34.7	9.55	47.8
40.0	38.5	96.3	20.4	51.1	16.9	42.3	14.9	37.2	21.1	52.7

1) geometric mean

LOQ = 0.1 µg a.s./L for processed samples

Table A7.4.1.3-24: Cell concentration data; cell number (× 10^4) and yield (= final cell number minus initial cell number of 10 000) dependent on mean measured concentrations of the test item and time; mean: arithmetic mean; cell number at test start: 10 000 cells/mL.

Test substance concentration (µg a.s./L), measured	Mean cell number (× 10^4)			
	0 h	*24 h*	*48 h*	*72 h*
Control	1	2.718	7.861	19.677
1.1	1	3.536	16.787	64.540
2.2	1	3.635	19.710	91.773
4.57	1	3.509	20.397	85.687
9.55	1	2.858	10.868	39.227
21.1	1	1.703	3.675	7.780
Temperature [°C]	23.0	22.9	23.0	22.9

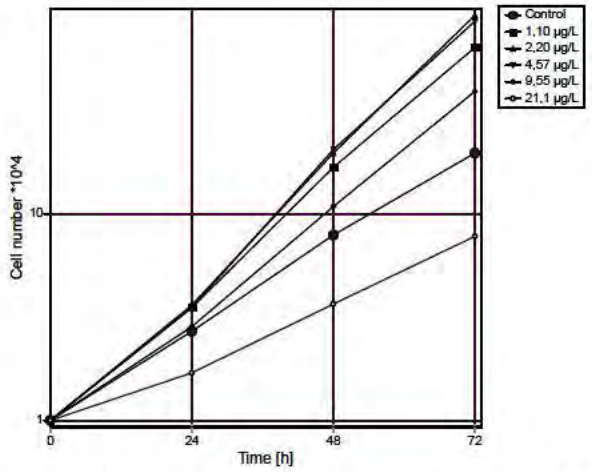

Figure A7.4.1.3- 3: Cell number (x 10^4) of *Pseudokirchneriella subcapitata* dependent on mean measured concentrations of the active substance Ampholyt 20.

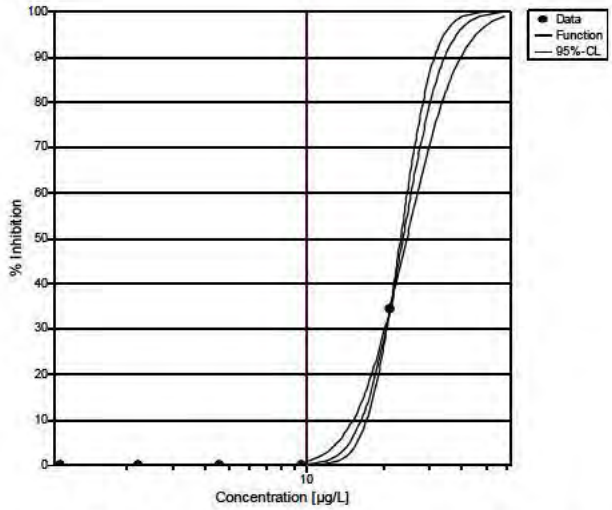

Figure A7.4.1.3- 4: Concentration-effect curve showing the influence of the mean measured concentrations of the test item (a.s.) on % inhibition of growth rate as observed after 72 h.

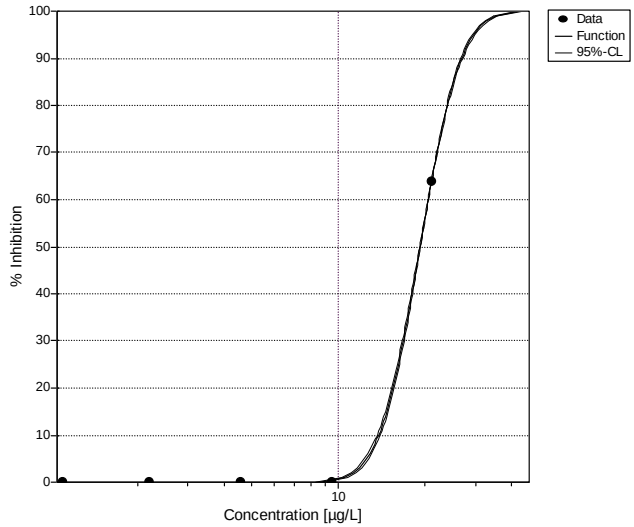

Figure A7.4.1.3- 5: Concentration-effect curve showing the influence of the mean measured concentrations of the test item (a.s.) on % inhibition of yield (cell number increase) as observed after 72 h.

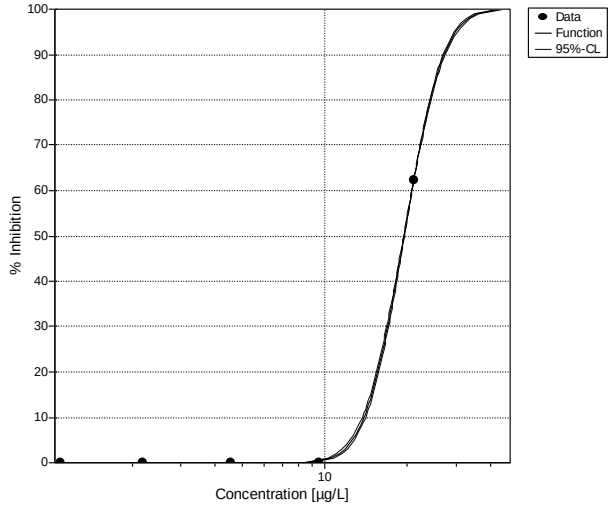

Figure A7.4.1.3- 6: Concentration-effect curve showing the influence of the mean measured concentrations of the test item (a.s.) on % decrease of biomass integral as observed after 72 h.

Table A7.4.1.3- 25: Percent inhibition of growth rate, yield and biomass integral by Ampholyt 20.

Test item [µg a.s./L]	% Inhibition of growth rate	% Inhibition of yield	% Inhibition of biomass
	0–72 h	0–72 h	0–72 h
1.10	–39.8	–240	–180
2.20	–51.8	–386	–272
4.57	–49.3	–353	–259
9.55	–22.8	–104	–72.1
21.1	34.5	63.7	62.2

Table A7.4.1.3- 26: Validity criteria for algal growth inhibition test according to OECD Guideline 201.

	Fulfilled	**Not fulfilled**
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	☑	
Evaluation of the sectional growth rates of the controls: The mean of the replicate coefficients of variations in the section-by-section growth rate of controls was 8.4 % (validity criterion ≤ 35 %)	☑	
The coefficient of variation of average specific growth rate in replicate control cultures during the whole test period was 2.5 % (validity criterion ≤ 7 %).	☑	

Section A7.4.1.4 **Inhibition to microbial activity (aquatic)**

Annex Point IIA 7.4 and IIIA 7.3

Official use only

Reference

Reference	**A7.4.1.4/01:** [redacted] (2002) Ampholyt 20/100 – determination of the inhibition of activated sludge respiration. Infracor GmbH, Marl, Germany, Report no. BH-02/05, April 22, 2002 (unpublished).
Data protection	Yes
Data owner	Goldschmidt GmbH
Companies with letter of access	No
Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

Guidelines and Quality Assurance

Guideline study	Yes OECD 209 (1984)
GLP	Yes
Deviations	No

Materials and Methods

Test material	Ampholyt 20/100 as given in Section A2.
Lot/Batch number	ES62403356
Specification	Ampholyt 20/100 as given in Section A2. The active substance as manufactured is obtained by the production process as a “product-by-process”, constituting a 20% (w/w) aqueous solution of the active matter. For the purpose of testing physicochemical properties, the material has exceptionally been lyophilised in order to obtain “pure” active substance, termed “Ampholyt 20/100”. Since the a.s. as manufactured consists of 20% active matter and 80% water, the question if lyophilised “Ampholyt 20/100” or the 20% product is subjected to biodegradability testing is considered to be of limited relevance for the reliability of the results.
Purity	99.4%
Composition of product	Not applicable
Further relevant properties	The test material is a multi-component substance as specified in Section A2.
Method of analysis	Not required.

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA 7.4 and IIIA 7.3

Preparation of TS solution for poorly soluble or volatile test substances	Not applicable
Reference substance	Yes: 3,5-dichlorophenol
Method of analysis for reference substance	None (not required)
Testing procedure	
Culture medium	Synthetic sewage feed, prepared in compliance with OECD 209.
Inoculum/test organism	Activated sludge, as described in Table A7.4.1.4- 2.
Test system	See Table A7.4.1.4- 3.
Test conditions	Details are presented in Table A7.4.1.4- 4.
Duration of the test	3 h
Test parameter	Inhibition of respiration
Analytical parameter	Oxygen concentration
Sampling	
Monitoring of TS concentration	No
Controls	Yes: 2 negative controls.
Statistics	EC_{50}: probit analysis

Results

Preliminary Test	Not performed
Concentration	
Effect data	
Results test substance	
Initial concentrations of test substance	5.0, 12.5, 32, 80, 200 and 500 mg/l
Actual concentrations of test substance	No analytical monitoring performed. Analytical monitoring is not necessary with this test protocol.
Growth curves	Not appropriate
Cell concentration data	Not appropriate
Concentration-response curve	A graph is presented in Figure A7.4.1.4- 1.
Effect data	EC_{20} = 11 mg/l EC_{50} = 22 mg/l (95 % CI = 19–25) EC_{80} = 44 mg/l

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA 7.4 and IIIA 7.3

Other observed effects	None
Results of controls	Please refer to Table A7.4.1.4- 5.
Test with reference substance	Performed
Concentrations	3.0, 7.5 and 19 mg/l
Results	For oxygen consumption data, see Table A7.4.1.4- 5. EC_{50} = 10.3 mg/l (reference substance)

Applicant's Summary and conclusion

Materials and methods	Inhibitory effects of Ampholyt 20/100 on microbial activity were tested by the activated sludge respiration inhibition test, following OECD guideline 209. No deviations from the guideline were reported.
Results and discussion	The test substance is not known to exhibit any properties that could have affected the outcome of the test.
EC_{20}	11 mg/l
EC_{50}	22 mg/l
EC_{80}	44 mg/l
Conclusion	The test fulfils the criteria of validity, since the two control respiration rates were within 15 % of each other and the EC_{50} of 3,5-dichlorophenol was in the accepted range 5 to 30 mg/l after 3 hours.
Reliability	1
Deficiencies	None

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	16th January 2013.
Materials and Methods	Test concentrations were not geometric means. Only 1 replicate per concentration tested, should have been at least triplicates. Lower concentrations should have been tested.
Results and discussion	Adopt applicants version.
Conclusion	Adopt applicants version.
Reliability	2
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.4.1.4- 1: Preparation of TS solution for poorly soluble or volatile test substances.

Criteria	Details
Dispersion	No
Vehicle	No
Concentration of vehicle	Not applicable
Vehicle control performed	Not applicable
Other procedures	None

Table A7.4.1.4- 2: Inoculum/ Test organisms.

Criteria	Details
Nature	Activated sludge
Species	Mixed species population
Strain	Not applicable
Source	Municipal wastewater plant
Sampling site	STP Marl-West, Germany
Laboratory culture	No
Method of cultivation	Not applicable
Preparation of inoculum for exposure	The sludge was extensively washed, resuspended and aerated
Pre-treatment	No
Initial cell concentration	4.24 g/l suspended solids (inoculum stock)

Table A7.4.1.4- 3: Test system.

Criteria	Details
Culturing apparatus	1000 ml Erlenmeyer flasks
Number of culture flasks/concentration	1
Aeration device	Clean, oil-free air, flow rate = 0.5 to 1 l/min
Measuring equipment	Microprocessor oximeter OXI 2000, WTW
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.4- 4: Test conditions.

Criteria	Details
Test temperature	18–20°C
pH	8.3–8.6
Aeration of dilution water	Yes
Suspended solids concentration	1.7 g/l in test medium

Table A7.4.1.4- 5: Respiration rates and percent inhibition values for Ampholyt 20/100, controls and the reference substance 3,5-dichlorophenol.

	3 h	
c [mg/l]	**Respiration rate [mg O_2/l × h]**	**% inhibition**
Test substance		
0.0 (Control 1)	29.26	–
0.0 (Control 2)	27.33	–
5	28.86	–2.0
12.5	24.36	13.9
32	4.67	83.5
80	0.85	97.0
200	0.45	98.5
500	0.07	99.8
Reference substance		
3	19.85	29.8
7.5	16.11	43.1
19	10.29	63.6

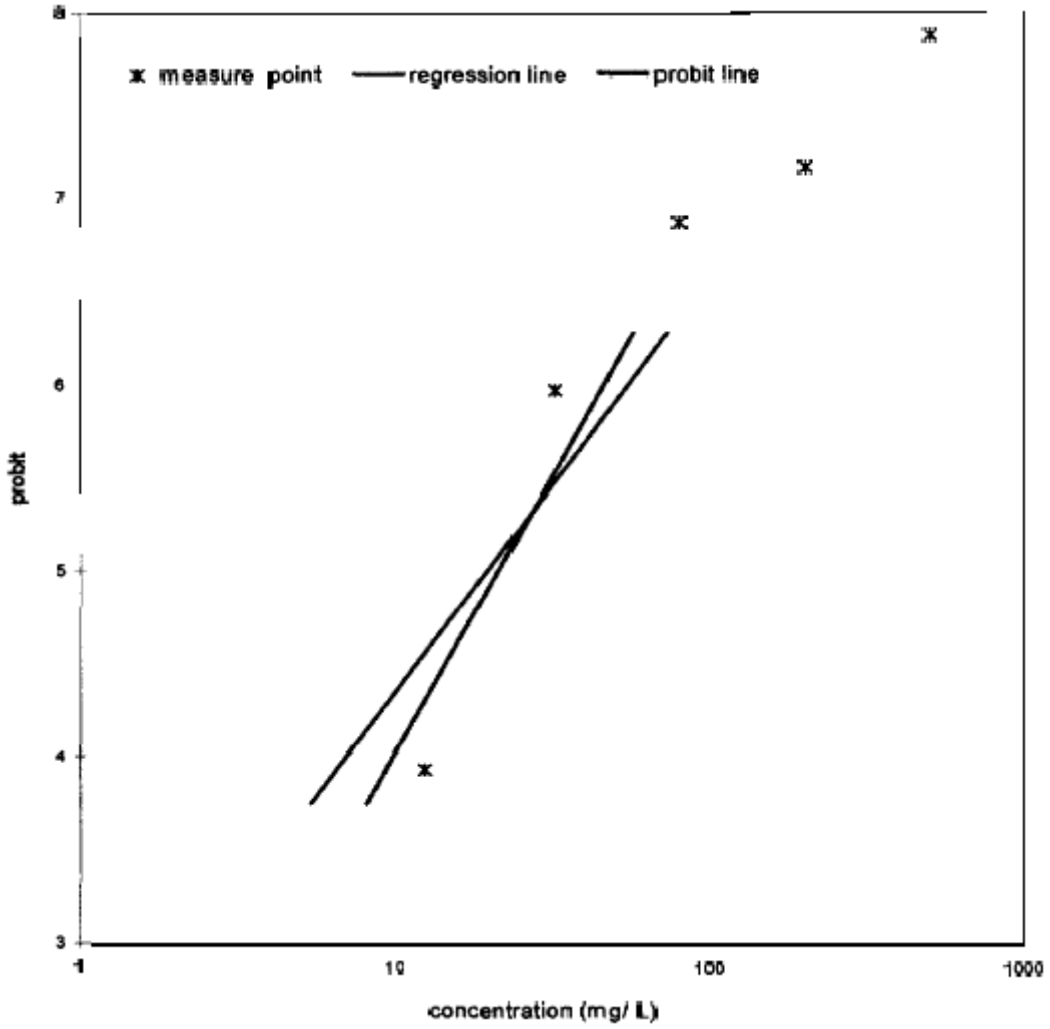

Figure A7.4.1.4- 1: Concentration-response curve for the respiration inhibition of activated sludge by Ampholyt 20/100.

Section A7.4.1.4 **Inhibition to microbial activity (aquatic)**

Annex Point IIA 7.4 and IIIA 7.3

Official use only

Reference

Reference	**A7.4.1.4/02:** [redacted] (2000): Activated sludge respiration inhibition test with TEGO 2000 (contact time 3 hours). NOTOX B.V., 's-Hertogenbosch, The Netherlands, unpublished report no. 291342, June 27, 2000.
Data protection	Yes
Data owner	Goldschmidt GmbH
Companies with letter of access	No
Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

Guidelines and Quality Assurance

Guideline study	Yes OECD 209 (1984) EEC Directive 67/548 (87/302)
GLP	Yes
Deviations	No

Materials and Methods

Test material	TEGO 2000, please refer to Section A2. 20% a. i. (aqueous solution, "product by process").
Lot/Batch number	S9B10B
Specification	TEGO 2000, as given in Section A2.
Purity	100 %
Composition of product	20% a. i. (aqueous solution, "product by process")
Further relevant properties	The a.i. is a multi-component substance as specified in Section A2. A specific method of analysis was not available at the time of test performance. The calculation of oxygen consumption in this test was determined for the amount of TEGO 2000, not for the active ingredient (being 20% of the amount of TEGO 2000). Therefore the endpoints for the active fraction of TEGO 2000 are calculated in this summary additionally concerning the active ingredient.
Method of analysis	None (not required)

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA 7.4 and IIIA 7.3

Preparation of TS solution for poorly soluble or volatile test substances	Not applicable
Reference substance	3,5–dichlorophenol
Method of analysis for reference substance	None A solution of 3,5-dichlorophenol (Aldrich, Cat. no. D 7,060-O, purity 97%) was prepared by dissolving 125.6 mg in 2.5 ml 1 N NaOH, diluting it to approximately 7.5 ml with Milli-Q water and adding under stirring 1 N H_2SO_4 to the point of incipient precipitation (approximately 2 ml of 1 N H_2SO_4 was required). Finally the solution was diluted to 250 ml with Milli-Q water. The pH was 7.6. Four concentrations were tested: 1.0, 3.2, 10 and 32 mg/l.
Testing procedure	
Culture medium	16 g peptone 11 g meat extract 3 g urea 0.7 g NaCl 0.4 g $CaCl_2$× 2 H_2O 0.2 g $MgSO_4$ × 7 H_2O 2.8 g K_2HPO_4 Dissolved in 1 l Milli-Q water and filtered. The pH was 7.0
Inoculum/test organism	Activated sludge, as described in Table A7.4.1.4- 6
Test system	Table A7.4.1.4-7
Test conditions	Table A7.4.1.4-8
Duration of the test	3 hours contact time during which aeration and stirring took place.
Test parameter	Oxygen consumption, inhibition of respiration
Analytical parameter	Oxygen concentration
Sampling	
Monitoring of TS concentration	No
Controls	Yes: 2 negative controls.
Statistics	Percent inhibition was calculated as prescribed by the test guideline. EC values were estimated using linear regression.

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA 7.4 and IIIA 7.3

Results

Preliminary Test	Not performed
Concentration	
Effect data	
Results test substance	
Initial concentrations of test substance	0, 32, 57, 101.8, 183.4, and 287.8 mg TEGO 2000/l. Since TEGO 2000 represents a 20% aqueous solution of the active substance, this corresponds to concentrations of 0, 6.4, 11.4, 20.36, 36.68, and 57.56 mg active substance. The content of water in TEGO 2000 is 80 %, therefore the growth inhibition should refer to the amount of a.i. and not to the product TEGO 2000.
Actual concentrations of test substance	No analytical monitoring performed. Analytical monitoring is not necessary with this test protocol.
Growth curves	Not appropriate
Cell concentration data	Not appropriate
Concentration-response curve	A graph is presented in Figure A7.4.1.4- 2
Effect data	EC_{10}: 87 mg/l TEGO 2000 EC_{20}: 120 mg/l TEGO 2000 EC_{50}: 280 mg/l TEGO 2000 Corresponding to: EC_{10}: 17.4 mg active ingredient/l EC_{20}: 24 mg active ingredient/l EC_{50}: 56 mg active ingredient/l
Other observed effects	None
Results of controls	Please refer to Table A7.4.1.4-9
Test with reference substance	Performed
Concentrations	Please refer to Table A7.4.1.4-9
Results	Interpolated from the regression line: y = 59.07 x + 7.04, please refer to Figure A7.4.1.4- 3.

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA 7.4 and IIIA 7.3

Applicant's Summary and conclusion

Materials and methods	The influence of TEGO 2000 on the respiration rate of activated sludge was investigated after a contact time of 3 hours. The study procedure was based on OECD Guideline No. 209, adopted April 4, 1984 and EEC Directive 67/548 amended November 18, 1987 (87/302), Part C, Publication No. L133, adopted May 30, 1988. TEGO 2000 was added directly and quantitatively to the test vessels. Weighed amounts were 16.0, 28.5, 50.9, 91.7 and 143.9 mg. The final volume of each test vessel was 500 ml resulting in test concentrations of 32.0, 57.0, 101.8, 183.4 and 287.8 mg/l TEGO 2000 (accordingly 0, 6.4, 11.4, 20.36, 36.68, and 57.56 mg active substance).
Results and discussion	Exposure of activated sludge bacteria to TEGO 2000 resulted in a concentration related change in oxygen consumption rates from 43 mg $O_2 \times l^{-1} \times h^{-1}$ at 32.0 mg/l TEGO 2000, the lowest concentration of active ingredient (6.4 mg a.i./l) tested to 15 mg $O_2 \times l^{-1} \times h^{-1}$ at 287.8 mg/l TEGO 2000 (57.56 mg a.i./l), the highest concentration tested. Relative to average control respiration rates this resulted in effects ranging from 18 % stimulation at 32.0 mg/l (6.4 mg a.i./l) to 59% inhibition at 287.8 mg/l (57.56 mg a.i./l). The EC_{50} was calculated to be 280 mg/l (56 mg a.i./l) with a 95% confidence interval ranging from 110 to 700 mg/l (22–140 mg a.i./l).
EC_{10}	87 mg/l TEGO 2000 (95% CI =39–200 mg/l) Corresponding to: 17.4 mg a.i./l (95% c 7.8–40)
EC_{20}	120 mg/l TEGO 2000 (95% CI =52–260 mg/l) Corresponding to: 24 mg a.i./l (95% CI = 10.4–52)
EC_{50}	280 mg/l TEGO 2000 Corresponding to: 56 mg a.i./l
Conclusion	The respiration rates of the controls were within 15% of each other. The EC_{50} of the reference substance, 3,5-dichlorophenol, was 5.3 mg/l. Therefore, the test was considered to be valid.
Reliability	1
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	21st January 2013
Materials and Methods	Experiment should have been carried out in triplicate, not 1 vessel per concentration.
Results and discussion	Adopt applicants version.
Conclusion	Adopt applicants version.
Reliability	2
Acceptability	Acceptable
Remarks	

	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.4.1.4- 6: Inoculum/ Test organisms.

Criteria	Details
Nature	Activated sludge
Species	Micro-organisms in activated sludge
Strain	Not applicable
Source	Municipal sewage treatment plant
Sampling site	Waterschap de Maaskant', 's-Hertogenbosch, the Netherlands.
Laboratoy culture	No
Method of cultivation	Not applicable
Preparation of inoculum for exposure	The sludge was coarsely sieved, washed and diluted with tap water. Before use the pH was checked (measured value: 7.1). A small amount of the sludge was weighed and dried at ca. 105°C to determine the amount of suspended solids (3.3 g/l of sludge, as used for the test).
Pre-treatment	The batch of sludge was used on subsequent days (maximum four days), therefore 50 ml of synthetic sewage feed was added to each litre of activated sludge at the end of each working day. The sludge was kept aerated at test temperature until use.
Initial cell concentration	Amount of suspended solids: 3.3 g/l of sludge

Table A7.4.1.4-7: Test system.

Criteria	Details
Culturing apparatus	All glass, 500 ml beakers and 300 ml oxygen bottles. 300 ml oxygen bottle: the flask was sealed with an oxygen electrode connected to a recorder, forcing the air out of the vessel.
Number of culture flasks/concentration	1
Aeration device	Oxygen consumption was measured and recorded for approximately 10 min. During measurement, the sample was not aerated but continuously stirred on a magnetic stirrer. The pH was determined in the remaining part of the reaction mixture.
Measuring equipment	Oxygen electrode (Tri Ox EO 200, WTW, FRG) supplied with a recorder (Kipp BD40)
Test performed in closed vessels due to significant volatility of TS	Not applicable

Table A7.4.1.4-8: Test conditions.

Criteria	Details
Test temperature	19 °C (test medium)
pH	Sludge before use: 7.1, pH-values measured during the test are given in Table A7.4.1.4-9.
Aeration of dilution water	Activated sludge (200 ml) was added to the synthetic sewage feed (16 ml) and an adequate amount of the test substance. The mixture was aerated in a 500 ml beaker during the contact time of 3 h, using a pipette as an aeration device.
Suspended solids concentration	

Table A7.4.1.4-9: pH, oxygen concentration at the start of measurement and the influence of 3,5-dichlorophenol and TEGO 2000 on the oxygen consumption of microbes in activated sludge and percentage inhibition in respiration rate.

Flask	Concentration reference/test substance (mg/l)	Initial oxygen conc. (≈ O_2/l/h)	Oxygen consumption (mg O_2/l/h)	Inhibition (%)	pH
c1	–	8.4	34	–	7.0
c2	–	8.7	36	–	7.0
mean c1+c2			35.0 (Δ6%)		
R1	1.0	8.0	33	6	7.2
R2	3.2	8.4.	22	37	7.5
R3	10	8.4	11	69	7.2
R4	32	8.6	2	94	7.2
c2	–	8.7	36	–	7.0
c3	–	8.3	37	–	7.0
mean c2+ c3			36.5 (Δ3%)		
T1	32.0 (6.4 a.i.)	7.7	43	–18	7.0
T2	57.0 (11.4 a.i.)	7.2	39	–7	7.0
T3	101.8 (20.36 a.i.)	7.3	34	7	7.0
T4		8.1	25	32	7.3
T5	183.4 (36.68 a.i.)	8.4	15	59	7.3
	287.8 (57.56 a.i.)				

c: control

R: Reference substance, 3.5-dichlorophenol

T: Test substance, TEGO 2000

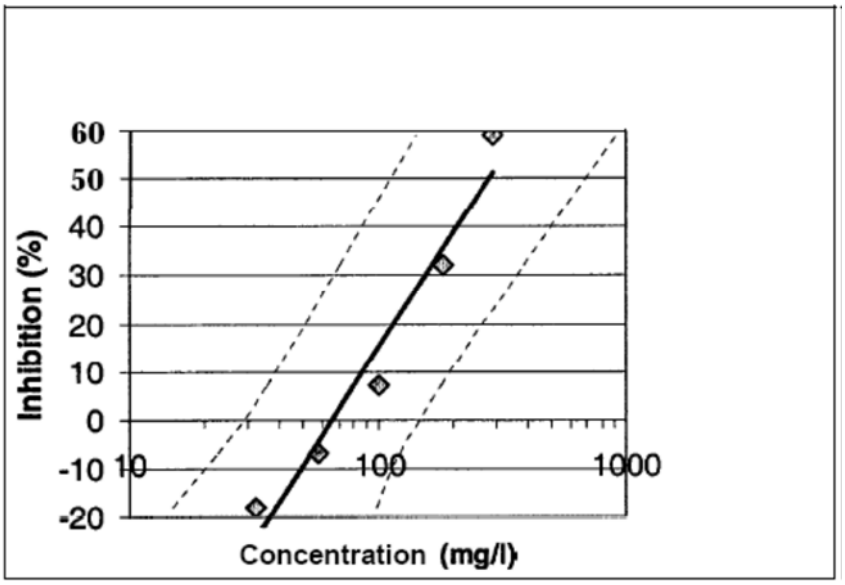

Figure A7.4.1.4- 2: Influence of TEGO 2000 (with a content of 20% a.i.) on the respiration rate of aerobic waste water (activated sludge) bacteria (values in % of the control) Dashed curves represent the 95 % confidence limits.

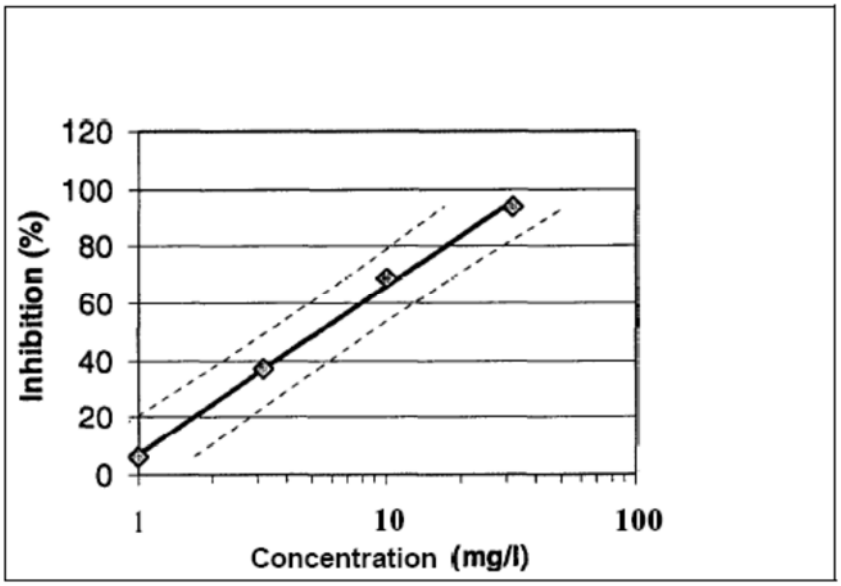

Figure A7.4.1.4- 3: Influence of 3,5-dichlorophenol on the respiration rate of aerobic wastewater (activated sludge) bacteria (values in % of the control). Dashed curves represent the 95 % confidence limits.

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA 7.4 and IIIA 7.3

The following reference is considered to contain additional information about inhibition to microbial activity and is thus presented in tabular format as supportive data:			
Reference	**Title**	**Method**	**Results**
A7.4.1.4/03: [redacted] (1992): Abwasser- und Peptonabbau-hemmungsuntersuchungen im Sapromat und modifizierter OECD-Bestätigungstest mit TEGOL 2000: Bayerische Landesanstalt für Wasserforschung, München, 1992 (unpublished)	„Waste Water and Peptone Degradation Inhibition Tests in the Sapromat and modified OECD Confirmatory Test with TEGOL 2000“	Sapromat inhibition tests with peptone and communal waste water (Degradation Inhibition with Tegol 2000): mechanically clarified communal waste water (source: Munich II, Germany) plus 100 mg/1 Peptone as feeding substrate Test substance: TEGO 2000 (containing 20% Ampholyt 20/100 in aqueous solution; Inoculum: activated sludge from a municipal STP; Test substance concentrations: 0.1, 1, 10 mg/l Analytical parameter: Oxygen concentration Test duration: 10 days GLP: No The study is poorly documented and thus of limited validity.	0.1 to 1 mg/1: no inhibition 10 mg/1: inhibitory or toxic effect

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	21st January 2013	
Materials and Methods	Adopt applicants version.	
Results and discussion	Adopt applicants version.	
Conclusion		
Reliability	3-4	
Acceptability	No	
Remarks	Not acceptable, results will not be entered in doc IIA	
	COMMENTS FROM ...	
Date		
Materials and Methods		
Results and discussion		
Conclusion		
Reliability		
Acceptability		
Remarks		

Section A7.4.2 **Bioconcentration in aquatic organisms**
Annex Point IIA7.5

Official use only

Reference

Reference	**A7.4.2/01:** [redacted] (2007) Estimation of the bioconcentration factor (BCF*fish*) of Ampholyt 20. EBRC Consulting GmbH, Hannover, Germany, Report no. DEG-20070628-01 June 28, 2007 (unpublished).
Data protection	Yes
Data owner	Goldschmidt GmbH
Companies with letter of access	No
Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

Guidelines and Quality Assurance

Guideline study	No A guideline for the estimation of bioconcentration does not exist. However, estimation was carried out in compliance with the procedure described in the TGD on Risk Assessment, for the estimation of bioconcentration in freshwater fish. The BCF_{fish} was estimated based on the octanol/water partition coefficient (P_{ow}).
GLP	Not applicable.
Deviations	Not applicable.

Materials and Methods

Test material	The test substance Ampholyt 20 is an amphoteric surfactant. It constitutes a complex mixture of partially carboxymethylated alkyl-propylene-diamines, obtained as a “product-by-process”.
Lot/Batch number	Not applicable (theoretical estimation)
Specification	Not applicable (theoretical estimation)
Purity	Not applicable (theoretical estimation)
Further relevant properties	None.
Method of analysis	Not applicable (theoretical estimation)
Reference substance	Not applicable
Method of analysis for reference substance	Not applicable
Testing/estimation procedure	

Section A7.4.2 Bioconcentration in aquatic organisms

Annex Point IIA7.5

Test system/ performance	Not applicable (theoretical estimation)
Estimation of bioconcentration	The estimation of the BCF_{fish} based on physical-chemical properties (log P_{ow}) as specified by the TGD on risk assessment. The partition coefficient (P_{ow}) of Ampholyt 20 has been calculated on the basis of a model calculation using the established QSAR (quantitative structure activity relationship) software (EpiSuite) for the various individual components of Ampholyt 20 (Horzella 2007, EBRC-No.: GOL-070524-01). Considering the relative proportions of the individual main components, the weighted mean log P_{ow} of Ampholyt 20 is given as 3.81. The original study on the partition coefficient is summarised in Section A3.9/02.

Results

Experimental data

Mortality/ behaviour	Not applicable.
Lipid content	Not applicable.
Concentrations of test material during test	Not applicable.
Bioconcentration factor (*BCF*)	Not applicable.
Uptake and depuration rate constants	Not applicable.
Depuration time	Not applicable.
Metabolites	Not applicable.
Other Observations	Not applicable.
Estimation of bioconcentration	$\log BCF_{fish} = 0.85 \times \log P_{ow} - 0.7$ BCF_{fish} = 345.54 l/kg wwt

Applicant's Summary and conclusion

Materials and methods	The bioconcentration factor in freshwater fish was estimated based on log P_{ow}, according to the procedure described in the TGD on Risk Assessment.
Results and discussion	Based on experimentally measured log P_{ow} values (reference A3.9/02) of 3.81, the predicted *BCF* in freshwater fish was estimated being BCF_{fish} = 345.54 l/kg wet fish
Conclusion	Bioconcentration in freshwater fish was estimated according to the method outlined in the TGD on Risk Assessment, based on GLP-conform data on physico-chemical properties. Therefore, the result is considered to be valid and reliable. The bioaccumulation potential of Ampholyt 20 is considered to be low.

Section A7.4.2 Bioconcentration in aquatic organisms

Annex Point IIA7.5

Reliability	0 (model calculation)
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	22nd January 2013
Materials and Methods	It is not possible to determine the terrestrial BCF for an ionisable substance using this calculation.
Results and discussion	Unacceptable
Conclusion	Unacceptable.
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	

Section A7.4.3.1 Prolonged toxicity to an appropriate species of fish

Annex Point IIIA13.2.1

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	According to the TNsG on data requirements, item 7.4.3.1, this test is usually not required since it does not add relevant information to the risk assessment. Since (i) a test on the growth rate of fish is presently ongoing which will finalised in due course (see section A7.4.3.2), waiving of the current data requirement is considered to be justified.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	22nd January 2013	
Evaluation of applicant's justification	Study A7.4.3.2 is sufficient to cover the data requirements for a long-term fish study.	
Conclusion	Accept justification.	
Remarks		
	COMMENTS FROM ...	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A7.4.3.2 **Effects on reproduction and growth rate on an appropriate species of fish**
Annex Point IIIA 13.2.2

Official use only

Reference

Reference	**A7.4.3.2/01:** [redacted] (2008): *Oncorhynchus mykiss*, juvenile growth test (OECD 215) flow-through exposure. Effect of Ampholyt 20 on the growth of juvenile rainbow trout. Fraunhofer-Institute for Molecular Biology and Applied Ecology (IME), Schmallenberg, Germany, Report no. EBR-013/4-63, March 06, 2008 (unpublished).
Data protection	Yes
Data owner	Goldschmidt GmbH
Companies with letter of access	No
Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

Guidelines and Quality Assurance

Guideline study	Yes OECD 215 (2000)
GLP	Yes
Deviations	No

Materials and Methods

Test material	Ampholyt 20 as given in Section A2.
Lot/Batch number	ES67345616
Specification	Ampholyt 20 as given in Section A2. The active substance as manufactured is obtained by the production process as a “product-by-process”, constituting a 20 % (w/w) aqueous solution of the active matter.
Purity	99 % w/w
Composition of product	Not applicable
Further relevant properties	The test material is a multi-component substance as specified in Section A2. Thus, analytical verification of test substance concentrations employed a lead substance concept, focussing on the C_{12}-alkyl compounds only.

Section A7.4.3.2 Effects on reproduction and growth rate on an appropriate species of fish
Annex Point IIIA 13.2.2

Method of analysis	The test item concentrations were analysed using HPLC-MS/MS. The limit of quantification for each lead compound was 0.1 µg/L. To assess the concentration of the test item Ampholyt 20, four "lead components" of the mixture were analysed, accounting together for 65.92 % of total active substance. The concentrations of the single lead compounds were summed up and extrapolated to the total active substance concentration. The method is identical to that reported in Section A4.2.
Preparation of TS solution for poorly soluble or volatile test substances	No
Reference substance	No
Method of analysis for reference substance	Not applicable.
Testing procedure	
Dilution water	Please refer to Table A7.4.3.2- 1.
Test organisms	Details on tested organisms are given in Table A7.4.3.2- 2.
Handling of embryos and larvae (OECD 215)	See Table A7.4.3.2- 2.
Test system	Please refer to Table A7.4.3.2- 3.
Test conditions	In each tank, dissolved oxygen (e.g. WTW Digital-Sauerstoff-Meßgerät Oxi Digi 550), pH (pH-Meter, e.g. WTW 535) and temperature (e.g. Digitalthermometer, Roth) were measured 5 days per week. Details are given in Table A7.4.3.2- 4.
Duration of the test	28 days
Test parameter	Mortality, wet weight, body length, and pseudo-specific growth rate
Examination/ Sampling	Behaviour and mortality: Daily Weight and length: Test start and after 14 days and 28 days Analytical samples: Test start and days 7, 14, 21, and 28
Monitoring of TS concentration	Yes, see 0 above.
Statistics	Since no effect on mortality, weight and pseudo-specific growth rate occurred, no statistical evaluation was performed.

Results

Range finding test	–
Concentration	–
Number/percentage of animals showing adverse effects	–
Nature of adverse effects	–

Section A7.4.3.2 Effects on reproduction and growth rate on an appropriate species of fish

Annex Point IIIA 13.2.2

Results test substance

Initial concentrations of test substance	23.5, 42.0, 93.5, 188 and 375 µg/L (corresponding to nominal concentrations of 4.7, 9.4, 18.8, 37.5 and 75.0 µg active substance/L) The samples taken from the test media of concentration 4 and 5 (37.5 and 75.0 µg a.s./L, respectively) were analyzed. Since no effect on juvenile growth occurred, no other concentration was necessary to verify. The samples of both analyzed treatments showed a comparable tendency of decrease in concentration and it could be assumed, that this is true for all treatments.
Actual concentrations of test substance	The nominal test concentrations of the a.s. were 4.69, 9.38, 18.75, 37.50, and 75.00 µg a.s./L. Due to a complete lack of adverse effects on juvenile growth, only the two highest test item concentrations (37.5 and 75 µg/L, nominal) were verified analytically. The arithmetic means of the measured concentrations of the four lead compounds were extrapolated to the total content of active substance. The recovery rate at 37.5 µg a.s./L (nominal) was 36.3 %, thus resulting in an actual the test item concentration of 13.6 µg a.s./L. At a nominal test item concentration of 75 µg a.s./L, 69.7 % of the lead compounds were recovered, corresponding to 52.3 µg a.s./L.
Effect data	No abnormal condition or behaviour was observed at any test concentration up to and including 52.3 µg/L (highest concentration tested, mean measured value). During the test no test item related mortality occurred. One fish was lost due to handling. At any concentration up to and including 52.3 µg a.s./L (measured), neither any significant effect nor any trend was observed. The fish weights increased by approx. 100 % every 14 d, thus the quality criterion for the controls (at least 50% in 28 d) was met even by all treatments. Regarding the pseudo-specific growth rate, no test item related effect was observed. The NOEC was established at ≥ 52.3 µg a.s./L (measured). Summary of the effect data is given in Table A7.4.3.2- 9

Section A7.4.3.2 Annex Point IIIA 13.2.2 — Effects on reproduction and growth rate on an appropriate species of fish

Concentration / response curve	Not applicable. A graph showing the concentration-mortality curve is not given since no fish died during the study.
Other effects	None
Results of controls	
Number/ percentage of animals showing adverse effects	No adverse effects on juvenile growth.
Nature of adverse effects	n.a.
Test with reference substance	Not performed
Concentrations	–
Results	–

Applicant's Summary and conclusion

Materials and methods	The effect of Ampholyt 20 on growth and survival of juvenile fish was tested according to OECD guideline 215 at nominal concentrations of 4.7, 9.4, 18.8, 37.5 and 75.0 µg active substance/L under flow-through conditions for 28 days.
Results and discussion	At mean measured concentrations up to and including 52.3 µg a.s./L, neither any significant effect nor any trend was observed. The fish grew well (203–223 % of start weight) during the test and fulfilled the control validity criteria of the OECD Guideline 215 at all treatment levels. As no effect was observed, the NOEC of the test item related to mean measured concentration was determined to be ≥ 52.3 µg a.s./L, representing ≥ 261.5 µg test item per litre.
NOEC	≥ 52.3 µg a.s./L (measured)
LOEC	Not determined
Conclusion	The test is considered to be valid (the validity-criteria are give in Table A7.4.3.2- 10.) According to the OECD guideline 215, for the whole batch of fish used in the test, the range in individual weights at the start should ideally be kept to within ± 10 % of the arithmetic mean weight and, should not exceed 25 %. In this study, two specimens allocated to treatment 4 and exceeded this threshold value, deviating by 26 % and 27 %, respectively. However, the length of both fish did not exceed ± 10 % of the arithmetic mean length. Furthermore, due to a lack of effects, the deviation is without influence on the integrity of the study. After completion of the validation of the analytical method, the samples taken from the test media of concentration 4 and 5 (37.5 and 75.0 µg a.s./L, nominal) were analyzed. The samples of both analyzed treatments showed similar pattern of decrease in concentration. Thus it can be safely assumed that this is representative for all other treatments. Since no effect on juvenile growth occurred, no other concentration was necessary to verify.
Reliability	1

Section A7.4.3.2 Annex Point IIIA 13.2.2	Effects on reproduction and growth rate on an appropriate species of fish	
Deficiencies	No Restriction of analytical verification to the two highest test concentrations is not considered to affect the reliability of the results. Since no effect on juvenile growth occurred at any test concentration, the NOEC is an adequately supported and fully valid endpoint. Moreover, since the NOEC is an unbounded value (no LOEC was observed), the endpoint is considered to be sufficiently robust for risk assessment.	

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	23rd January 2013	
Materials and Methods	Adopt applicants version.	
Results and discussion	Adopt applicants version.	
Conclusion	Adopt applicants version.	
Reliability	1	
Acceptability	Acceptable.	
Remarks		
	COMMENTS FROM ...	
Date		
Materials and Methods		
Results and discussion		
Conclusion		
Reliability		
Acceptability		
Remarks		

Table A7.4.3.2- 1: Dilution water.

Criteria	Details
Source	Cu-free tap water
Salinity	–
Alcalinity	0.7–1.1 mmol/L
Hardness	0.7–1.0 mmol total hardness
pH	8.03 (please refer to Table A7.4.3.2- 8)
Oxygen content	80 % oxygen saturation
Conductance	161.3–183.7
Holding water different from dilution water	No

Table A7.4.3.2- 2: Test organisms.

Criteria	Details
Species/strain	Juvenile rainbow trout (*Oncorhynchus mykiss*)
Source	Eye point stage eggs were obtained from the NRW Landesanstalt für Fischerei (governmental fisheries agency), Albaum, Germany on March 22, 2007 and further bred in the laboratory of the Fh-IME (test facility)
Wild caught	No
Age/size	5 cm ± 1 cm; 3 g ± 1 g
Kind of food	Trout food (Trouvit Alleinfuttermittel, Milkivit, D- 86664 Burgheim)
Amount of food	All fish were weighed before test start. A feeding rate of 4 % of body weight was adjusted, divided into two equal portions per day. The fish were weighed again on day 14 of the test to recalculate and adjust the feeding rate, and on day 28 to obtain the results of growth.
Feeding frequency	Twice per day.
Post-hatch transfer time	Rainbow trout fingerlings were bred at 12.0 °C under flow-through conditions in the test facility; test fish were held at least two weeks prior to the test under conditions of water quality and illumination similar to those used in the test, i.e. at 14 ± 2 °C and at a photoperiod of 14 h/d. They were fed a minimum ratio of 2 % body weight per day; the fish were reared in water of the same quality as used in the test (purified drinking water).
Feeding of animals during test	Yes
Treatment for disease within 2 weeks preceding test	Not stated, only fish without visible abnormalities were used in the study.

Table A7.4.3.2- 3: Test system.

Criteria	Details
Test type	Flow-through
Renewal of test solution	Flow rate of about 2.5 L/h (daily turnover of about 5 volumes
Volume of test vessels	10 L
Volume/animal	1 L
Number of animals/vessel	10
Number of vessels/concentration	2
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.3.2- 4: Test conditions.

Criteria	Details
Test temperature	13.3–14.9 °C; for details please refer to Table A7.4.3.2-6.
Dissolved oxygen	65–98 %; for details please refer to Table A7.4.3.2- 7.
pH	7.5–8.1; for details please refer to Table A7.4.3.2- 8.
Adjustment of pH	No
Aeration of dilution water	Yes
Quality/Intensity of irradation	The light intensity did not exceed 1000 lux
Photoperiod	16 h photoperiod daily during the test period.

Table A7.4.3.2- 5: Measured concentrations and mean of the four “Lead components” of the active substance Ampholyt 20 in the "flow-through *Oncorhynchus mykiss*, juvenile growth test" test media (75.0 µg a.s./L, nominal).

Lead compound	Nominal concentration [µg/L]	Measured [µg/L] (% nom.)					
		Test start	7 d	14 d	21 d	28 d	Mean
[redacted]	26.71	29.95 (112.1)	15.76 (59.0)	16.29 (61.0)	9.34 (35.0)	3. 74 (14.0)	15.01 (56.21)
[redacted]	7.8	9.58 (122.8)	8.03 (103.0)	15.73 (201.7)	16.75 (214.7)	6.51 (83.5)	11.32 (145.12)
[redacted]	11.70	6.32 (54.0)	4.53 (38.7)	7.94 (67.9)	8.77 (75.0)	3.36 (28.7)	6.19 (52.86)
[redacted]	3.23	1.65 (51.1)	1.40 (43.3)	2.63 (81.5)	3.14 (97.1)	0.98 (30.4)	1.96 (60.71)
Sum of lead components	49.44	47.49 (96.0)	29.73 (60.1)	42.60 (86.2)	37.99 (76.8)	14.59 (29.5)	34.48 (69.74)
Extrapolated to total a.s.	75.0						52.3 (69.7)

Table A7.4.3.2- 6: Temperature [°C] in the test tanks throughout the test. a.s. = active substance; n. = nominal; SD = standard deviation; m.m. = mean measured; Concentrations given as nominal and mean measured concentrations, where appropriate.

	a.s. concentration [µg/L]					
	Control	**4.69 n.**	**9.38 n.**	**18.75 n.**	**37.50 n.**	**75.00 n. (52.3 m m.)**
Min	13.5	13.4	13.5	13.3	13.4	13.4
Max	14.9	14.6	14.5	14.3	14.3	14.6
Mean	14.3	14.1	14.0	13.9	13.9	14.2
SD	0.3	0.3	0.3	0.2	0.3	0.3

Table A7.4.3.2- 7: Oxygen saturation of the test media (%).Values of the test tanks throughout the test (28 days). a.s. = active substance; n. = nominal; SD = standard deviation; m.m. = mean measured; Concentrations given as nominal and mean measured concentrations, where appropriate.

	a.s. concentration [µg/L]					
	Control	**4.69 n.**	**9.38 n.**	**18.75 n.**	**37.50 n.**	**75.00 n. (52.3 m m.)**
Min	70	70	65	66	66	70
Max	98	91	95	90	95	98
Mean	81	81	81	80	81	80
SD	7	6	8	6	5	7

Table A7.4.3.2- 8: pH of the test media in the test tanks throughout the test (28 days). a.s. = active substance; n. = nominal; SD = standard deviation; m.m. = mean measured; Concentrations given as nominal and mean measured concentrations, where appropriate.

	a.s. concentration [µg/L]					
	Control	**4.69 n.**	**9.38 n.**	**18.75 n.**	**37.50 n.**	**75.00 n. (52.3 m m.)**
Min	7.57	7.54	7.52	7.51	7.58	7.57
Max	8.08	8.04	8.05	8.08	8.09	8.09
Mean	7.8	7.8	7.8	7.7	7.7	7.7
SD	0.2	0.1	0.1	0.1	0.1	0.1

Table A7.4.3.2- 9: Effect data: Fish weights (g) and length (cm) at test start, day 14 and at test end (28 days). (n per vessel = 10). a.s. = active substance; SD = standard deviation; n. = nominal; m m. = mean measured; concentrations given as nominal and mean measured concentrations; mean individual pseudo-specific r_3 (tank-average specific r_2) growth

rate from day 0 to 28. (n per vessel = 10); no significant deviation occurred, compared to the control.

		a.s. concentration [µg/L]					
		Control	**4.69 n.**	**9.38 n.**	**18.75 n.**	**37.50 n.**	**75.00 n. (52.3 m m.)**
Fish weights	Mean 28 d	8.33 g (SD 1.64)	8.69 g (SD 1.13)	8.5 g (SD 2.11)	8.44 g (SD 1.32)	8.7 g (SD 1.89)	8.49 g (SD 2.09)
	Mean gain* 0–28 d	5.75 g	5.95 g	5.7 g	5.75 g	5.83 g	5.78 g
Fish length	Mean 28 d	8.9 cm (SD 0.5)	8.8 cm (SD 0.5)	8.8 cm (SD 0.6)	8.7 cm (SD 0.5)	8.9 cm (SD 0.6)	8.7 cm (SD 0.7)
	Mean gain* 0–28 d	2.5	2.5	2.4	2.4	2.5	2.4
Pseudo-specific growth rate	Mean (=r_2)	4.2 (SD 0.7)	4.3 (SD 0.5)	4.2 (SD 0.9)	4.2 (SD 0.6)	4.3 (SD 0.8)	4.2 (SD 0.8)

No fish showed any clinical sign of intoxication

* pseudo-specific weight/length gain as individual weight after 28 d / mean weight at test start

Table A7.4.3.2- 10: Validity criteria for fish test according to OECD Guideline 215.

	Fulfilled	**Not fulfilled**
Concentration of dissolved oxygen > 60% saturation throughout the test	☑	
Difference of water temperature < 1° C between test chambers at any time during test; temperature within a range of 2° C of the temperature for specific test species	☑	
Mortality of control animals <10%	☑	
Increase of fish weight sufficient for detection of the minimum variation of growth rate considered as significant	☑	
Criteria for poorly soluble test substances	Not applicable	

Section A7.4.3.3.1 Bioaccumulation in an appropriate species of fish

Annex Point IIIA 13.2.3

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	According to the physico-chemical properties, especially the estimated average partition coefficient of log P_{ow} = 3.81 (A3.9/02) and the predicted bioconcentration factor BCF_{fish} of 345.54 l/kg wet fish (A7.4.2), there is no indication of a significant bioaccumulation potential of Ampholyt 20. Thus, the conduct of a specific bioaccumulation study in fish is not considered to be required.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	5th march 2013	
Evaluation of applicant's justification	Justification unacceptable. The log p_{ow} cannot be used to determine the BCF potential of Ampholyt 20. See position paper Ampholyt Log D calculation in section IIIA and section IIA Ecotoxicology Non-compartment specific effects relevant to the food chain (secondary poisoning). The BCF_{fish} of 345.54 L/Kg is also unacceptable. The CA decision is that Ampholyt does not have a potential to bioaccmulate in fish.	
Conclusion		
Remarks		
	COMMENTS FROM ...	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A7.4.3.3.2 Bioaccumulation in an appropriate invertebrate species

Annex Point IIIA 13.2.3

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	According to the physico-chemical properties, especially in view of the estimated average partition coefficient of log P_{ow} = 3.81 (A3.9/02) and the predicted bioconcentration factor $BCF_{earthworm}$ of 78.32 l/kg wwt (A7.5.5.1), there is no indication of a significant bioaccumulation potential of Ampholyt 20. An experimental study would only be appropriate if direct release to marine or brackish water is likely, as outlined in the TNsG on data requirements. However, according to the envisaged use pattern of Ampholyt 20 (surface disinfectant, product types 2, 3, 4) direct release of the active substance to surface waters is very unlikely. Thus, the conduct of a specific bioaccumulation study in invertebrates is not considered to be required.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	23rd January 2013
Evaluation of applicant's justification	Justification acceptable.
Conclusion	Acceptable.
Remarks	
	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A7.4.3.4
Annex Point IIIA 13.2.4

Effects on reproduction and growth rate with an invertebrate species

Official use only

Reference

Reference	**A7.4.3.4/01:** [redacted] (2007): *Daphnia magna*, Reproduction test (OECD 211) Semi-static exposure. Effect of Ampholyt 20 on the reproduction of *Daphnia magna*. Report no. EBR-013/4-21, Fraunhofer-Institute for Molecular Biology and Applied Ecology (IME), Schmallenberg, Germany, July 19, 2007 (unpublished). **A7.4.3.4/02:** [redacted] (2008): Amendment no. 1 to study report *Daphnia magna*, reproduction test (OECD 211) semi-static exposure. Effect of Ampholyt 20 on the reproduction of *Daphnia magna*. Recalculation of effect values based on analytically verified test concentrations. Report no. EBR-013/4-21, Fraunhofer-Institute for Molecular Biology and Applied Ecology (IME), Schmallenberg, Germany, amendment dated March 20, 2008 (unpublished).
Data protection	Yes
Data owner	Goldschmidt GmbH
Companies with letter of access	No
Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I of Directive 98/8/EC.

Guidelines and Quality Assurance

Guideline study	Yes OECD 211 (1998)
GLP	Yes
Deviations	No

Materials and Methods

Test material	As given in Section A2.
Lot/Batch number	ES67345616
Specification	As given in Section A2. The active substance as manufactured is obtained by the production process as a “product-by-process”, constituting a 20 % (w/w) aqueous solution of the active matter.
Purity	20 % (w/w) aqueous solution of pure active matter (99 % w/w)
Composition of product	20 % a.i. (aqueous solution, “product by process”)
Further relevant properties	The a.i. is a multi-component substance as specified in Section A2.

Section A7.4.3.4 Effects on reproduction and growth rate with an invertebrate species
Annex Point IIIA 13.2.4

Method of analysis	Since Ampholyt 20 consists of a variety of components most of which are not commercially available as analytical standard, four "lead components" of the mixture were analysed as given in detail in Section A4.2. To assess the concentration of the test item Ampholyt 20, the four "lead components" of the mixture were analysed, accounting together for 65.92 % of total active substance. The concentrations of the single lead compounds were summed up and extrapolated to the total active substance concentration. The test item concentrations were analysed using HPLC-MS/MS. The limit of quantification for each lead compound was 0.1 µg/L. For details of the analytical method see Section A4.2.
Preparation of TS solution for poorly soluble or volatile test substances	Not applicable
Reference substance	No
Method of analysis for reference substance	–
Testing procedure	
Dilution water	Purified drinking water: Please refer to Table A7.4.3.4- 1.
Test organisms	*Daphnia magna*. Please refer to Table A7.4.3.4-2.
Handling of offspring	At the end of the test, the total number of living offspring produced per parent animal alive was assessed.
Test system	Semistatic. Please refer to Table A7.4.3.4-3.
Test conditions	Please refer to Table A7.4.3.4-4.
Duration of the test	21 days
Test parameter	Survival, body growth, physical/pathological symptoms and changes in behaviour, Reproduction and population growth
Examination/ Sampling	Daily
Monitoring of TS concentration	Yes The concentrations of the test item Ampholyt 20 were assessed by chemical analysis of aliquots taken from fresh and aged test solutions. Fresh medium was sampled at renewal (day 1, 3, 5, 7, 11, 13, 14, 17, and 19) and aged media immediately prior to renewal (day 2, 4, 6, 8, 12, 14, 15, 18, and 20). The walls of representative test vessels of the lowest, middle and highest test item concentration were eluted after 24 h aging at three renewals with an organic solvent-mixture (125 mL methanol + 125 mL dichloromethane + 500 µL trifluoroacetic acid) to elute potentially adsorbed test item. All samples were stored frozen at -18 °C (± 2 °C) until analysis.

Section A7.4.3.4
Annex Point IIIA 13.2.4

Effects on reproduction and growth rate with an invertebrate species

Statistics	Calculations were performed with the computer software ToxRat Professional version 2.09 (release 08.11.2006) by ToxRat® Solutions GmbH. A NOEC was calculated using ANOVA followed by Williams' test or an appropriate non-parametric test suggested by the ToxRat program. Test results showing a concentration-response relationship were analysed by regression to determine the EC_{50} including the 95 % confidence interval as well as the EC_{10} using probit analysis assuming log-normal distribution of the values.

Results

Range finding test	Not performed
Concentration	–
Number/percentage of animals showing adverse effects	–
Nature of adverse effects	–
Results test substance	
Initial concentrations of test substance	The test item was Ampholyt 20. The nominal concentration in the test containers with test item was 4.6, 11.5, 28.75, 72.0, and 180.0 µg test item per litre, representing 0.92, 2.30, 5.75, 14.40, and 36.00 µg active substance (nominal) per litre.
Actual concentrations of test substance	In representative fresh media the measured concentrations of the test item were in the range of 39% to 334% of nominal, independent of the concentrations. During the 24 h renewal period the test item concentrations decreased to levels of 8 % to 170 % of nominal. The data are summarised in Table A7.4.3.4- 5. Due to the decrease of the exposure concentrations during the test period, the time weighted means (TWM) of the measured concentrations were used for the evaluation of the effect concentrations. The TWM of the treatments were calculated to be 0.78, 2.28, 2.39, 11.35, and 27.48 µg a.s./L (85.3, 99.2, 41.5, 78.8, and 76.3 % of nominal).

Section A7.4.3.4 Effects on reproduction and growth rate with an invertebrate species

Annex Point IIIA 13.2.4

Effect data	Neither adult mortality nor any sublethal effects were observed up to a concentration of 2.4 µg a.s./L (TWM, NOEC). The EC_{10}, EC_{20}, and EC_{50} were estimated at 3.1, 4.7, and 10.6 µg a.s./L (TWM), respectively. Adult body length exhibited no significant differences between treatments up to the highest concentration tested (NOEC ≥ 27.5 µg a.s./L (TWM)). All surviving specimens gave the impression of healthy condition. Survival, growth and reproduction data are given in Table A7.4.3.4-6 and Table A7.4.3.4-7. Data about parental length at day 21 are given in Table A7.4.3.4-8. Age at first brood was between 9.0 and 10.5 days across treatment level (NOEC ≥ 27.5 µg a.s./L (TWM)). The cumulative number of offspring per parent animal ranged from 28.0 to 67.6 across treatment levels, showing an apparent concentration-response relationship. NOEC = 2.3 µg a.s./L, EC_{10} = 3.4 µg a.s./L, EC_{20} = 6.8 µg a.s./L, and EC_{50} 24.6 µg a.s./L (TWM)
Concentration / response curve	Please refer to Figure A7.4.3.4- 1, Figure A7.4.3.4- 2, and Figure A7.4.3.4- 3.
Other effects	None
Results of controls	The results of controls are included in the tables below.
Test with reference substance	Not required
Concentrations	–
Results	–

Applicant's Summary and conclusion

Materials and methods	The influence of Ampholyt 20 on the reproduction of *Daphnia magna* was investigated. A 21-day semi-static exposure to Ampholyt 20 at nominal concentrations of 0.92, 2.30, 5.75, 14.40, and 36.00 µg a.s./L, with daily renewal of the test solutions was conducted according to OECD guideline 211. Untreated control replicates were run in parallel. Each treatment group consisted of 10 replicates with one daphnid each (individual exposure). Effects on growth (adult length at test termination) and reproductive performance were investigated. Samples of fresh and aged test solutions were analysed for test item concentrations. Since the nominal concentrations varied by more than 20 %, the biological effects were re-evaluated based on mean measured concentrations in reference A7.4.3.4/02. Due to a decrease of test item concentration during the renewal period, the time weighted mean values (TWM) for each treatment level were calculated. The respective TWM were 0.78, 2.28, 2.39, 11.35, and 27.48 µg a.s./L (85.3, 99.2, 41.5, 78.8, and 76.3 % of nominal).

Section A7.4.3.4 Annex Point IIIA 13.2.4 — Effects on reproduction and growth rate with an invertebrate species

Results and discussion	The mean values for the different test endpoints per treatment level are listed in Table A7.4.3.4-6. The NOEC, EC_{10}, EC_{20}, and EC_{50} values of the biological endpoints are summarised in Table A7.4.3.4-7. All effect concentrations are given as time weighted mean concentrations of the a.s. of Ampholyt 20.
NOEC	2.4 µg a.s./L for parental survival 2.3 µg a.s./L for cumulative offspring per *Daphnia* 2.3 µg a.s./L for intrinsic rate of increase ≥ 27.5 µg a.s./L for age at the first brood and growth (length on day 21)
LOEC	n.d.
EC_{50} (EC_x)	Please refer to Table A7.4.3.4-7.
Conclusion	The validity criteria are considered as fulfilled (please refer to Table A7.4.3.4-9).
Reliability	1
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	24th January 2013
Materials and Methods	Adopt applicants version
Results and discussion	Adopt applicants version
Conclusion	Adopt applicants version
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.4.3.4- 1: Dilution water.

Criteria	Details
Source	Purified drinking water was used as holding- and dilution water. The purification included filtration with activated charcoal, passage through a limestone column, and aeration. To avoid copper contamination, plastic water pipes are used for the testing facilities. The following water chemistry data are recorded regularly in the testing facility and are reported: pH, conductivity, dissolved oxygen content, content of nitrate, nitrite, ammonium (NH_4^+), phosphate, calcium, magnesium, total hardness, alkalinity, DOC content, content of metals (copper, iron, manganese and zinc).
Salinity	Not reported
Hardness	Ca-hardness: 0.4–0.5 mmol/l Mg-hardness: 0.2–0.4 mmol/l
pH	Not reported
Ca / Mg ratio	Not reported
Na / K ratio	Not reported
Oxygen content	Not reported
Conductance	Not reported
TOC	0
Holding water different from dilution water	No

Table A7.4.3.4-2: Test organisms.

Criteria	Details
Strain / Clone	*Daphnia magna* (clone V)
Source	German Federal Environment Agency, Institut für Wasser-, Boden- und Lufthygiene. Specimens used in the test were bred in the laboratory of the Fraunhofer IME.
Age	*Daphnia magna* less than 24 h old
Breeding method	Adult Daphnia, at least 3 weeks old, were separated from the stock population by sieving. Batches of 30 to 50 animals are held at room temperature in ca. 1.8 L dilution water for one week. During this week the daphnids were fed daily with an algal suspension (*Desmodesmus subspicatus*) and LiquizellR (HOBBY). The water was changed once per week. Newborn Daphnia were separated by sieving, the first generation was discarded.
Kind of food	Suspensions of unicellular alga *Desmodesmus subspicatus*.
Amount of food	Algae growing in the log-phase were centrifuged and the pellet was re-suspended in a few mL of medium. 30 mL of this suspension was given to 1 L Daphnia medium.
Feeding frequency	Daily
Pre-treatment	Not reported
Feeding of animals during test	Yes. The daphnids were fed during the test with suspensions of unicellular alga *Desmodesmus subspicatus*. The content of food in the test suspensions, measured as turbidity at 758 nm, increased during the test period of 21 days from about 7 mg C/L equivalents to 15 mg C/L equivalents.

Table A7.4.3.4-3: Test system.

Criteria	Details
Test type	Semistatic exposure
Renewal of test solution	Daily
Volume of test vessels	50 ml
Volume/animal	50 ml
Number of animals/vessel	1
Number of vessels/concentration	10
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.3.4-4: Test conditions.

Criteria	Details
Test temperature	19.7–20.4 °C
Dissolved oxygen	Oxygen saturation of the overlaying water as measured as nominal values throughout the test [mg/L]: Min: 7.6, max: 9.0 Beginning: 7.8–8.0 End: 8.4–8.7
pH	pH (start): 7.8 During the test: 7.6 (min) – 8.4 (max.) At test end: 8.0–8.3
Adjustment of pH	No
Aeration of dilution water	No
Quality/intensity of irradiation	The light intensity did not exceed 15–20 µE/(m² × s) or 1125–1500 lx. Measurements conducted during test: min. 508 lx to max. 591 lx
Photoperiod	Light/dark cycle of 16/8 hours

Table A7.4.3.4- 5: Time weighted mean (TWM) of the measured concentrations of the four lead compounds (sum) extrapolated to total active substance (a.s.) and recovery rate.

Nominal conc. a.s. (µg/L)	Nominal conc. lead compounds (µg/L)	TWM active substance (a.s.) (µg/L)	TWM sum of lead compounds (µg/L)	TWM Recovery rate (%)
0.92	0.61	0.78	0.52	85.3
2.30	1.52	2.28	1.50	99.2
5.75	3.79	2.39	1.57	41.5
14.4	9.49	11.35	7.48	78.8
36.0	23.73	27.48	18.11	76.3

Table A7.4.3.4-6: Survival, growth and reproduction data. Concentrations are given as nominal concentrations. SD = standard deviation. Number of D. magna per concentration: n = 10.

Concentration (nominal a.s.)	TWM active substance (a.s.)	Parental survival	Growth (length on day 21)	Age at first brood	Cumulative offspring per female	Intrinsic rate of increase
(µg/L)	(µg/L)	(%)	Mean ± SD (mm)	Mean ± SD (days)	Mean ± SD (Ind.)	Mean ± SD (Ind./day)
Control		100	4.73 ± 0.28	9.4 ± 0.7	67.6 ± 9.5	0.302 ± 0.030
0.92	0.8	100	4.70 ± 0.37	9.0 ± 0.8	65.7 ± 7.2	0.309 ± 0.026
2.30	2.3	100	4.67 ± 0.35	9.2 ± 0.7	62.9 ± 9.1	0.292 ± 0.024
5.75	2.4	90	4.62 ± 0.31	10.1 ± 1.1	57.4 ± 13.2	0.277 ± 0.025
14.4	11.4	40	4.35 ± 0.36	10.5 ± 1.6	53.5 ± 13.0	0.238 ± 0.038
36.0	27.5	20	4.63 ± 0.28	10.1 ± 2.1	28.0 ± 8.5	0.204 ± 0.013

Table A7.4.3.4-7: Effect summary table. NOEC and EC values [µg a.s./L]. NOEC = No observed effect concentration; EC = Effect concentration; CL = Confidence level; n.d. = not determined due to mathematical reasons; Effect concentrations given as time weighted mean (a.s.).

Concentration	Parental survival	Growth (length on day 21)	Age at first brood	Cumulative offspring per female	Intrinsic rate of increase
EC_{50} (95% CL)	10.6 (6.5–17.1)	n.d. (n.d.)	> 27.5 (n.d.)	24.6 (11.3–>27.5)	> 27.5 (n.d.)
EC_{20} (95% CL)	4.7 (2.8–8.2)	n.d. (n.d.)	> 27.5 (n.d.)	6.8 (0–14.0)	11.5 (7.4–16.2)
EC_{10} (95% CL)	3.1 (1.6–6.0)	n.d. (n.d.)	24.2 (n.d.)	3.4 (n.d.–8.5)	4.4 (1.7–7.0)
NOEC	2.4	≥ 27.5	≥ 27.5	2.3	2.30

Table A7.4.3.4-8: Parental lengths at day 21 [mm]. a.s. = Active substance; Concentrations given as nominal values and TWM (time weighted mean); † = dead at test end.

Replicate	Control	0.92 µg a.s./L (TWM: 0.8 µg a.s./L)	2.30 µg a.s./L (TWM: 2.3 µg a.s./L)	5.75 µg a.s./L (TWM: 2.4 µg a.s./L)	14.4 µg a.s./L (TWM: 11.4 µg a.s./L)	36.0 µg a.s./L (TWM: 27.5 µg a.s./L)
1	4.78	4.33	4.48	4.49	4.45	†
2	4.93	4.75	4.13	5.18	3.75	†
3	4.22	4.07	4.23	4.25	4.59	†
4	5.07	5.04	4.68	4.44	4.33	†
5	4.34	4.37	4.89	4.35	†	4.43
6	4.66	4.86	5.17	4.56	†	†
7	4.79	4.56	4.98	†	†	†
8	5.03	4.82	4.36	4.56	4.64	4.83
9	4.91	4.88	4.81	4.73	†	†
10	4.56	5.34	4.99	5.03	†	†

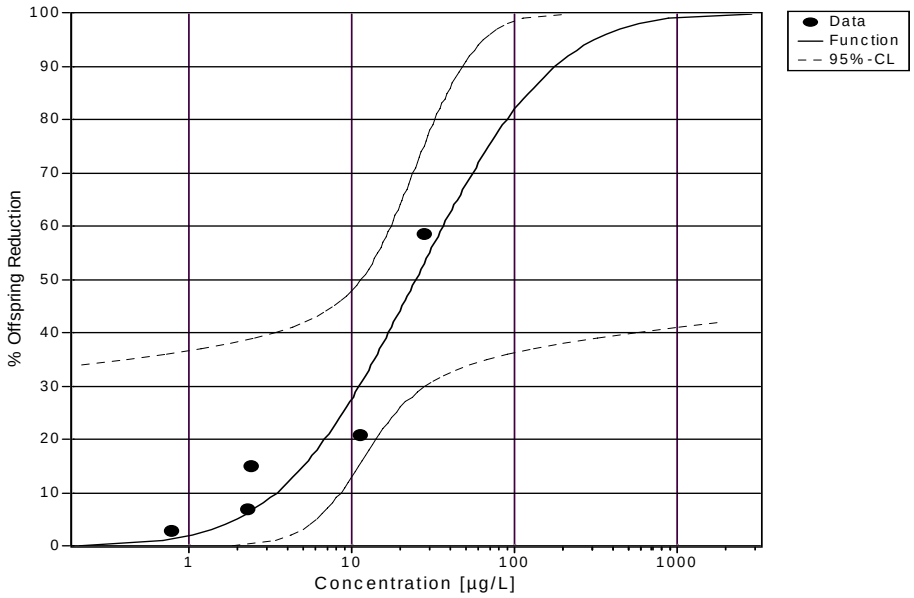

Figure A7.4.3.4- 1: Concentration-effect curve showing the influence of the test item on cumulative offspring of survivors of the introduced *Daphnia magna* as observed after 21 d.

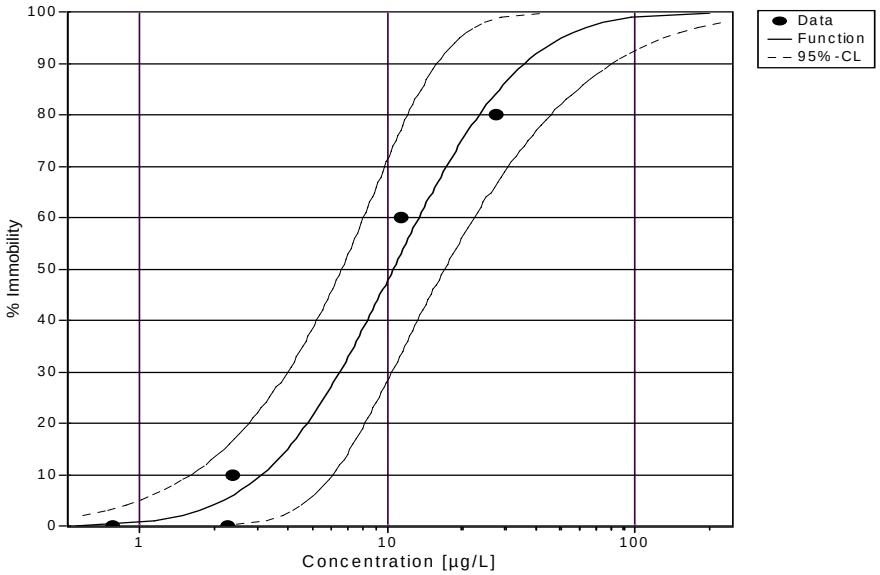

Figure A7.4.3.4- 2: Concentration-effect curve showing the influence of the test item on mobility of the introduced Daphnia magna as observed after 21 d.

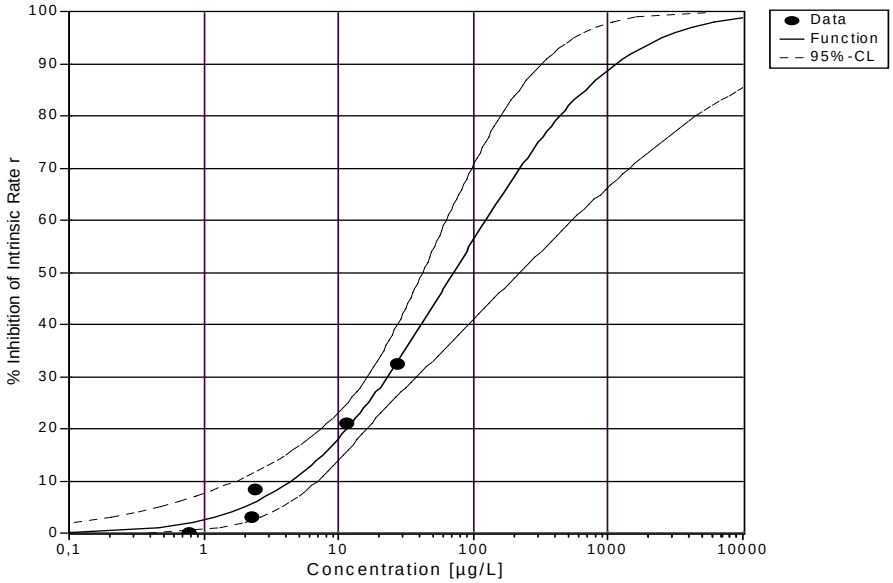

Figure A7.4.3.4- 3: Concentration-effect curve showing the influence of the test item on intrinsic rate r of the introduced Daphnia magna as observed after 21 d.

Table A7.4.3.4-9: Validity criteria for invertebrate reproduction test according to OECD Guideline 211.

	Fulfilled	Not fulfilled
Mortality of parent animals < 20% at test termination	☑	
Mean number of live offspring produced per parent animal surviving at test termination ≥ 60	☑	
Survival in the control (100%) was above 80%	☑	
The daphnids in the control started to reproduce until day 9	☑	
The coefficient of variation for the mean number of offspring in the controls (5 %) was below 25 %	☑	

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA 13.2.4

JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA 13.2.4

Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [X]**
Limited exposure [X]	**Other justification [ ]**
Detailed justification:	According to the physico-chemical properties, especially in view of the estimated average partition coefficient of log P_{ow} = 3.81 (A3.9/02) and the predicted solids-water partition coefficient for sediment of 50.1 l/kg (see Document II-A, chapter 4.1.1.3), sediment is not considered as a significant target compartment. Experimental studies on sediment organisms would only be appropriate if direct release to marine, brackish or fresh water is likely. However, according to the envisaged use pattern (surface disinfectant, product types 2, 3, 4, without any direct release to marine waters) direct release of the active substance to surface waters is very unlikely. Instead, any releases of disinfectants are primarily directed to the sewage treatment plant. The substance has been shown to be readily biodegradable (Section A7.1.1.2.1). Thus, particular risks for sediment dwelling organisms are not expected and the conduct of specific studies is not considered to be required.
Undertaking of intended data submission [ ]	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	4th March 2013
Evaluation of applicant's justification	The log D, which is more suitable for estimating the partition coefficient of Ampholyte 20 is ≤ 1.5. The suspended matter-water partition coefficient is 2.5 x 10^4 m^3/m^3. No direct release to sediment organisms, therefore justification for non inclusion of a sediment dwelling orgmanisms study is accepted by CA.
Conclusion	Justification acceptable.
Remarks	
	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A7.4.3.5.2 Aquatic plant toxicity

Annex Point IIIA 13.2.4

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	The substance has been shown to be readily biodegradable (Section A7.1.1.2.1). Direct exposure of the aquatic environment to Ampholyt 20 is not foreseen, since any releases of disinfectants to the environment are primarily directed to the sewerage. It is therefore considered to be feasible to base the risk assessment for the aquatic environment on the available conventional ecotoxicological studies (chronic toxicity to fish, *Daphnia* and algae). Accordingly, specific risks for aquatic plants are not expected and testing for aquatic plant toxicity is not considered to be required.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	4th March 2013.	
Evaluation of applicant's justification	Justification acceptable.	
Conclusion	Acceptable.	
Remarks		
	COMMENTS FROM ...	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A7.5.1.1 **Inhibition of microbial activity (terrestrial)**
Annex Point IIA 7.4

Official use only

Reference

Reference	**A7.5.1.1/01:** [redacted] (2007): Soil Microorganisms – Effects of Ampholyt 20 on Nitrogen and Carbon Transformation. Fraunhofer-Institute for Molecular Biology and Applied Ecology (IME), Schmallenberg, Germany. Report No. EBR-013/3-35, September 10, 2007 (unpublished).
Data protection	Yes
Data owner	Goldschmidt GmbH
Companies with letter of access	No
Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

Guidelines and Quality Assurance

Guideline study	Yes OECD guidelines 216 and 217 (2000) EU Methods C.21 and C.22.
GLP	Yes
Deviations	No

Materials and Methods

Test material	As given in Section A2.
Lot/Batch number	ES67345616
Specification	As given in Section A2. The active substance as manufactured is obtained as a “product-by-process”, constituting a 20 % (w/w) aqueous solution of the active matter.
Purity	20 % (w/w) aqueous solution of pure active matter (99 % w/w)
Composition of product	20 % a.i. (aqueous solution, “product by process”)
Further relevant properties	The a.i. is a multi-component substance as specified in Section A2. The test substance is hydrolytically stable (water solubility ≥ 200 g/L) and the vapour pressure is 1.9×10^{-4} Pa (see section A3).
Method of analysis	Not required according to the test guidelines.
Reference substance	According to the test guidelines 216 and 217, no reference item is required.

Section A7.5.1.1 Inhibition of microbial activity (terrestrial)

Annex Point IIA 7.4

Method of analysis for reference substance	Not applicable
Testing procedure	
Soil sample	Please refer to Table A7.5.1.1-1.
Test system	Please refer to Table A7.5.1.1-2.
Application of TS	Please refer to Table A7.5.1.1- 3.
Test conditions	Please refer to Table A7.5.1.1-4.
Test parameter	Inhibition of microbial activity by Ampholyt 20: Nitrogen transformation: Nitrate concentration in soil was determined after amending the soil by addition of powdered plant material (lucerne-grass-green meal) at a plant-soil ratio of 5 g/kg soil (dry mass) as a natural nitrogen source. Carbon transformation: Short-term respiration (glucose induced respiration rates) was determined as oxygen consumption.
Analytical parameter	Nitrogen transformation: Nitrate: photometrically (Spectroquant® NOVA 400) Nitrate was extracted from soil by shaking samples (10 g dry mass) with 0.1 M KCl solution at a ratio of 5 mL of KCl solution per gram dry weight for 60 minutes at 150 rpm. The mixtures were filtered and the liquid phases were photometrically analysed for nitrate. Carbon transformation: Glucose-induced respiration rates: oxygen consumption (Sapromat® Voith Inc.) The glucose amended soil samples were incubated in an apparatus for continuous measurement of respiration rates (Sapromat® Voith Inc.) at 20 ± 2 °C. The oxygen consumed was measured for 12 consecutive hours.
Duration of the test	28 days
Sampling	At test start and after 28 days of incubation
Monitoring of TS concentration	No
Controls	Blank control without test substance (4 replicates).
Statistics	NOEC: William's test (one-sided) Concentration-effect relationship: Probit-analysis assuming log-normal distribution (Computer software ToxRat Professional version 2.09 (release 30.10.2005), ToxRat® Solutions GmbH)

Results

Range finding test	Not reported
Concentration	
Effect data	

Section A7.5.1.1 Inhibition of microbial activity (terrestrial)

Annex Point IIA 7.4

Results test substance

Initial concentrations of test substance	The nominal test item concentrations in the test containers were 8.2, 20.5, 51.2, 128, 320, 800, 2000, and 5000 mg test item/kg, corresponding to 1.64, 4.10, 10.24, 25.6, 64, 160, 400, and 1000 mg a.s./kg dry mass, respectively.
Actual concentrations of test substance	Nominal. The actual concentration of active substance was not measured during the test. This is not required according to the test guideline. In view of the nature of the test item (aqueous solution), homogeneous mixing with the soil may be safely assumed.
Growth curves	Not applicable.
Cell concentration data	Not applicable.
Concentration/ response curve	Graphs are given in Figure A7.5.1.1- 1 and Figure A7.5.1.1- 2.
Effect data	The results of the nitrate measurement are presented as mean values in Table A7.5.1.1- 5. The results of the short-term respiration measurements are presented as mean values in Table A7.5.1.1- 6. The effect data are given in Table A7.5.1.1- 7.
Other observed effects	None
Results of controls	Data for the controls without test substance are included in the Table A7.5.1.1- 5 and Table A7.5.1.1- 6.
Test with reference substance	Not performed
Concentrations	–
Results	–

Applicant's Summary and conclusion

Materials and methods	The effect of Ampholyt 20 on nitrogen and carbon transformation by soil microorganisms was investigated according to the OECD guidelines 216 and 217. The test item Ampholyt 20 was incorporated into loamy sand soil at various concentrations at test start. The effects on nitrogen transformation and short-term respiration were determined 28 days after start of incubation.
Results and discussion	With respect to the nitrogen transformation capacity and short-term respiration, no inhibitory effect of Ampholyt 20 was observed. A hypothetical EC_{50} for both, nitrogen and carbon transformation would be > 1000 mg a.s. per kg dry soil.
NOEC	≥ 1000 mg a.s./kg dry soil.
EC_{10}	Not determined
EC_{50}	> 1000 mg a.s./kg dry soil. (95% CL not determined for mathematical reasons)

Section A7.5.1.1 Inhibition of microbial activity (terrestrial)

Annex Point IIA 7.4

Conclusion	Ampholyt 20 did not show any negative effects on nitrogen transformation and carbon transformation in the tested loamy sand field soil up to the limit test concentration of 1000 mg a.s./kg dry soil (NOEC). The study is considered to be valid without restrictions. The variation between replicate control samples was less than ±15 % at day 28.
Reliability	1
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	31st January 2013
Materials and Methods	Adopt applicants version.
Results and discussion	According to the discussion following submission of the CAR, the NOEC had to be recalculated. Considering the corrected NO_3 values on day 28, clear inhibitory effects can be seen at 160 mg a.i./kg. Therefore, the NOEC would be 64 mg a.i./kg dw.
Conclusion	Adopt corrected version.
Reliability	1
Acceptability	Acceptable.
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.5.1.1-1: Soil sample.

Criteria	Details
Nature	Soil sample (RefeSol-01-A, batch IME-01)
Sampling site:	Agricultural field with no culture
Geographical reference on the sampling site	D-57377 Schmallenberg, Germany
Data on the history of the site	No plant protection product for at least one year prior to sampling and no organic or mineral fertilisation six or three months prior to sampling
Use pattern	Agricultural soil
Depth of sampling [cm]	Not stated
Sand / Silt / Clay content [%]	71 / 24 / 5
pH	5.7
Organic carbon content [%]	0.93
Nitrogen content [%]	0.09
Cation exchange capacity [mmol/kg]	37.9
Initial microbial biomass	145 mg C/kg dry mass soil), calculated from respiration activity)
Reference of methods	Not stated
Collection / storage of samples	The soil (RefeSol-01-A, batch IME-01) was taken from the reference soils at the Fraunhofer Institute for Molecular Biology and Applied Ecology (IME) on June 15, 2007. After sieving to 2 mm on June 18, 2007 the samples were stored until June 26, 2007 (date of application) at room temperatures in the dark. The soil moisture was maintained at 40–60 % of WHC.
Preparation of inoculum for exposure	The soil conditioning to 20°C started on June 18, 2007.
Pre-treatment	No

Table A7.5.1.1-2: Test system.

Criteria	Details
Culturing apparatus	Test containers
Number of vessels/concentration	3 replicates per test concentration. Blank controls: 4 replicates.
Aeration device	No additional aeration
Measuring equipment	Nitrate: Photometer Spectroquant® NOVA 400 Glucose-induced respiration rates: continuous measurement of respiration rates: oxygen consumption (Sapromat® Voith Inc.)
Test performed in closed vessels	Not appropriate

Table A7.5.1.1- 3: Application of test substance.

Criteria	Details
Application procedure	The quantity of test item required to obtain the desired concentrations was filled up to 60 mL with water (the amount of water needed to adjust the test substrate to 52–58 % water holding capacity), added to the soil, mixed thoroughly and placed into a test container.
Carrier	Water
Concentration of liquid carrier [% v/v]	See above
Liquid carrier control	Not applicable
Other procedures	No

Table A7.5.1.1-4: Test conditions.

Criteria	Details
Organic substrate	Nitrogen transformation: Lucerne-grass-green meal at a plant-soil ratio of 5 g plant per kilogram of soil (dry mass) Carbon transformation: Glucose at a rate of 4000 mg/kg dry weight using a glucose/talcum mixture to optimize a homogeneous distribution in the soil
Incubation temperature	20 ± 2 °C
Soil moisture	55 % of the WHCmax with a range of 3 %; controlled weekly by weighing; adjustment using deionised water as needed
Method of soil incubation	individual subsamples
Aeration	No

Table A7.5.1.1- 5: Mean nitrate content [mg/kg] and deviation from control [%]; a.s. = active substance; concentrations given as nominal concentration per kg dry soil; significant differences were not detected compared to control (Williams t-test, p = 0.05, one-sided).

	Control	1.64 mg a.s./kg	4.10 mg a.s./kg	10.2 mg a.s./kg	25.6 mg a.s./kg	64 mg a.s./kg	160 mg a.s./kg	400 mg a.s./kg	1000 mg a.s./kg
Nitrate [mg/kg]									
Test start	78.8	95.0	95.0	121.7	123.3	105.0	106.7	110.0	93.3
Day 28	202.5	190.0	210.0	203.5	226.7	240.0	243.3	296.7	428.3
Deviation [%]		–6.2	3.7	0.4	11.9	18.5	20.2	46.5	111.5

The amount of nitrate arising from the amount of nitrogen supplied at each test concentration must be subtracted from the total nitrate amount at 28 d. This is possible to do conservatively by assuming:1 mg N is equivalent to 4.4 mg NO3. The results are shown below

mg a.s/L	NO3 mg/kg	NO3 from test substance	Corr.	% deviation

	Day 28		NO_3 mg/kg Day 28	
Cont	202.5		202.5	
1.64	190.0	0.93	189	-6.6
4.10	210.0	2.3	207.7	+2.5
10.2	203.5	5.8	197.7	-2.3
25.6	226.7	14.6	212.1	+ 4.7
64	240.0	36.6	203.4	+ 0.4
160	243.3	91.5	151.8	- 25
400	296.7	228.8	67.9	-66
1000	428.3	572	0	- 100

Considering the corrected NO_3 values on day 28, clear inhibitory effects can be seen at 160 mg a.i./kg. Therefore, the NOEC would be 64 mg a.i./kg dw.

Table A7.5.1.1- 6: Mean short-term respiration rate ([mg O_2/(kg × h)] and deviation from control [%]; a.s. = active substance; SIR = Substrate induced respiration; concentrations given as nominal concentration per kg dry soil; significant differences were not detected compared to control (Williams' t-test, p = 0.05, one-sided).

	Control	**1.64 mg a.s./kg**	**4.10 mg a.s./kg**	**10.2 mg a.s./kg**	**25.6 mg a.s./kg**	**64 mg a.s./kg**	**160 mg a.s./kg**	**400 mg a.s./kg**	**1000 mg a.s./kg**
SIR [mg O_2/(kg × h)]									
Test start	5.17	4.51	5.42	6.06	7.15	6.07	5.91	7.34	5.82
Day 28	3.88	4.13	3.11	3.02	3.35	3.52	3.97	6.87	10.22
Deviation [%]		6.4	–19.8	–22.2	–13.7	–9.3	2.3	77.1	163.4

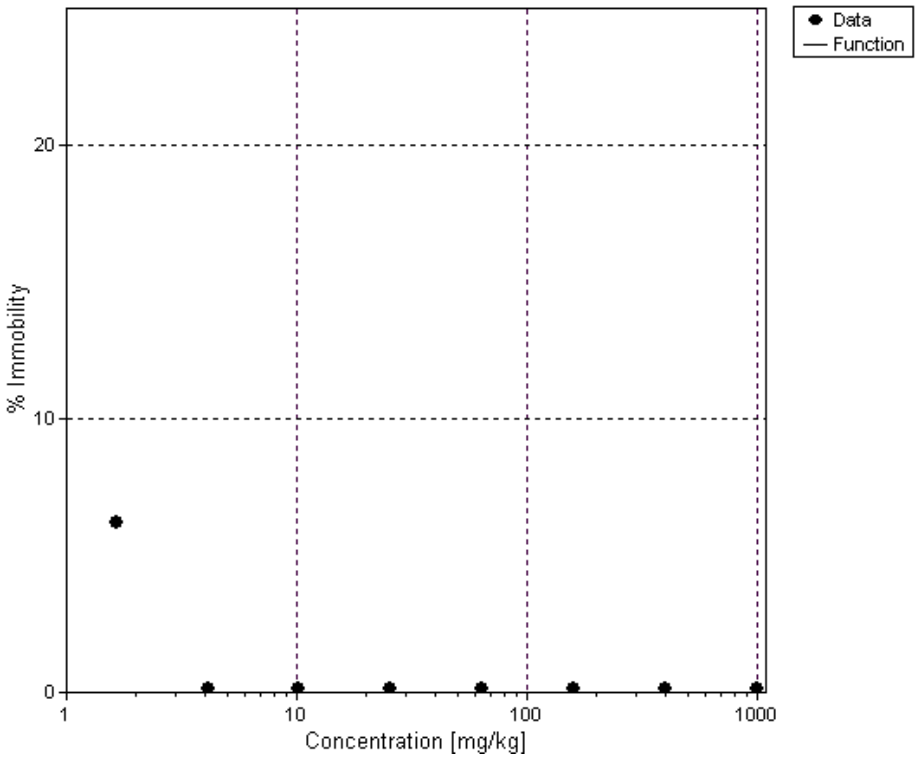

Figure A7.5.1.1- 1: Effect related to control at test end, Nitrogen transformation. Effect on nitrogen amount. No calculation of response curve possible.

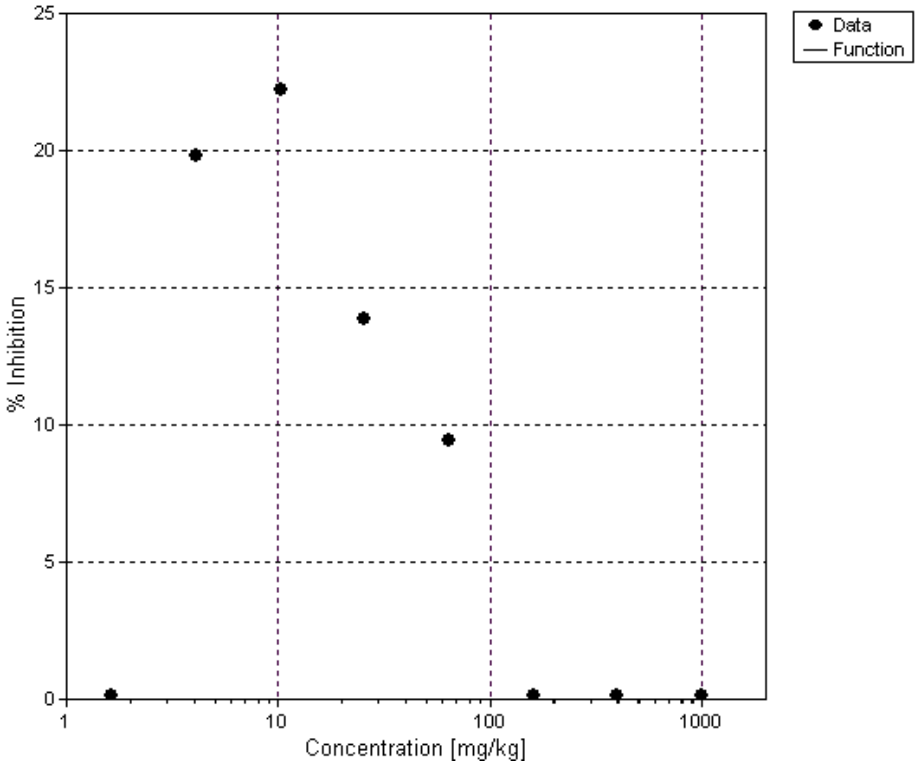

Figure A7.5.1.1- 2: Effect related to control at test end, Carbon transformation. Effect on substrate induced respiration (SIR) No calculation of response curve possible

Table A7.5.1.1- 7: NOECs and EC_X values [mg a.s./kg dry mass] for nitrogen and carbon transformation. NOEC = No observed effect concentration (effect as inhibition); EC = Effect concentration where x % inhibition occurred against control; CL = Confidence limits; n.d. = not determined due to mathematical reasons; a.s. = active substance; Concentrations given as nominal concentration per kg dry mass.

	Nitrogen transformation	**Carbon transformation**
NOEC	≥ 1000	≥ 1000
EC_{10} (95 % CL) [mg/kg]	> 1000	> 1000
EC_{20} (95 % CL) [mg/kg]	> 1000	> 1000
EC_{50} (95 % CL) [mg/kg]	> 1000 (n.d.)	> 1000 (n.d.)

Section A7.5.1.2 **Earthworm, acute toxicity test**
Annex Point IIIA 13.3.2

Official use only

Reference

Reference	**A7.5.1.2/01:** [redacted] (2007): Earthworm Acute Toxicity Test – Acute Toxicity of Ampholyt 20 on *Eisenia fetida*. Fraunhofer-Institute for Molecular Biology and Applied Ecology (IME), Schmallenberg, Germany, Report No. EBR-013/3-08, September 04, 2007 (unpublished).
Data protection	Yes
Data owner	Goldschmidt GmbH
Companies with letter of access	No
Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

Guidelines and Quality Assurance

Guideline study	Yes OECD guideline 207 (1984) EC method C.8
GLP	Yes
Deviations	No

Materials and Methods

Test material	As given in Section A2.
Lot/Batch number	ES67345616
Specification	As given in Section A2. The active substance as manufactured is obtained as a “product-by-process”, constituting a 20% (w/w) aqueous solution of the active matter.
Purity	20 % (w/w) aqueous solution of pure active matter (99 % w/w)
Composition of product	20 % a.i. (aqueous solution, “product by process”)
Further relevant properties	The a.i. is a multi-component substance as specified in Section A2.
Method of analysis	None
Reference substance	2-chloroacetamide A test with the reference substance is regularly performed at the test facility once a year, thus fulfilling guideline recommendations.
Method of analysis for reference substance	Not required according to the test guideline.

Section A7.5.1.2 Earthworm, acute toxicity test

Annex Point IIIA 13.3.2

Testing procedure

Preparation of the test substance	Not applicable.
Application of the test substance	The quantity of test item required to obtain the desired concentrations was added on a weight basis into a glass beaker, diluted in about 20 mL water, applied to the required amount of test substrate per concentration and mixed thoroughly. Subsequently, 753 g test substrate (adjusted to 60 % water holding capacity) was added to each replicate.
Test organisms	*Eisenia fetida*, as detailed in Table A7.5.1.2- 1.
Test system	See Table A7.5.1.2- 2.
Test conditions	See Table A7.5.1.2- 3.
Test duration	14 d
Test parameter	Mortality, weight change at test end.
Examination	At the beginning and at the end of the test.
Monitoring of TS concentration	Not required according to the test guideline.
Statistics	The percent weight change of the worms was calculated as an absolute value and in comparison to the control. Potential effects of the solvent were evaluated by comparison of blank and solvent control using Student's t-test; the NOEC regarding weight change was determined using Williams' test, and the LC_{50} was estimated by probit analysis of log-normal distributed data using the computer software ToxRat Professional version 2.09 (release 30.10.2005) by ToxRat® Solutions GmbH.

Results

Filter paper test	Not performed.
Concentration	Not applicable.
Number/ percentage of animals showing adverse effects	Not applicable.
Nature of adverse effects	Not applicable.
Soil test	
Initial concentrations of test substance	1.64, 4.10, 10.24, 25.6, 64.0, 160, 400, and 1000 mg active substance per kg dry soil.
Effect data (Mortality)	No mortality up to 1000 mg/kg; for details see Table A7.5.1.2- 5.
Concentration / effect curve	Please refer to Figure A7.5.1.2- 1.
Other effects	Weight change of the worms at test end are documented in Table A7.5.1.2- 6 and graphically presented in Figure A7.5.1.2- 1.
Results of controls	The results of controls are included in the tables below.
Test with reference substance	Tests with the reference substance 2-chloroacetamide are performed once a year.

Section A7.5.1.2 Earthworm, acute toxicity test

Annex Point IIIA 13.3.2

Concentrations	80 mg/kg	
Results	Weight change:	Control: –3 %
		80 mg 2-chloroacetamide/kg: all specimens dead
	Mortality:	Control: 0 %
		80 mg 2-chloroacetamide/kg: 100 %
		related to control: 100 %
	EC/LC_{50} values:	Weight change: 20–80 mg/kg
		Mortality: 20–80 mg/kg

Applicant's Summary and conclusion

Materials and methods

The effect of Ampholyt 20 on survival of adult earthworms of the species *Eisenia fetida* was investigated according to EC method C.8. The worms were placed in a defined artificial soil substrate containing the test item in nominal concentrations of 1.64, 4.1, 10.24, 25.6, 64, 160, 400, and 1000 mg active substance per kg dry soil. The test item was incorporated into the test soil at the beginning of the experiment, and the effects on biomass and mortality were determined after 14 days.

Results and discussion

There was no mortality, neither in the controls nor at any test concentration up to the limit test concentration of 1000 mg a.s./kg dry soil. The NOEC for the tested species was found to be ≥ 1000 mg a.s./kg dry soil.

There was no statistically significant influence of Ampholyt 20 on body weight change up to the limit test concentration of 1000 mg a.s./kg dry soil. The NOEC for the tested species was found to be ≥ 1000 mg a.s./kg dry soil.

LC_0	> 1000 mg a.s./kg dry soil
LC_{50}	> 1000 mg a.s./kg dry soil (CI n.d.)
LC_{100}	≥ 1000 mg a.s./kg dry soil (CI n.d.)

Conclusion

Ampholyt 20 is not acutely toxic to *Eisenia fetida* under the given test conditions. NOEC and EC_{50} are ≥ 1000 mg a.s./kg dry soil (see validity criteria summarized Table A7.5.1.2- 8).

Reliability 1

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	31st January 2013	
Materials and Methods	Total carbon and total organic carbon of soil not stated.	
Results and discussion	Adopt applicants version.	
Conclusion	Acceptable	
Reliability	1	
Acceptability	Acceptable	
Remarks		
	COMMENTS FROM ...	
Date		
Materials and Methods		
Results and discussion		
Conclusion		
Reliability		
Acceptability		
Remarks		

Table A7.5.1.2- 1: Test organisms.

Criteria	Details
Species	*Eisenia fetida andrei* (Annelida, Oligochaeta)
Source of the initial stock	Regenwurmfarm Tacke, Klosterdiek 61, 46325 Borken. Specimens used in the test were bred in the laboratory of the test facility
Culturing techniques	The breeding conditions followed SOP V3-255/02. Worms were bred in 1:1 mixtures of cow manure and Sphagnum peat (dry mass basis) at 20 °C ± 2 °C.
Age/weight	2–3 months old, with a clitellum, and a wet mass between 300 mg and 600 mg.
Pre-treatment	The worms were conditioned in the artificial soil for 6 days before start of the test.

Table A7.5.1.2- 2: Test system.

Criteria	Details
Artificial soil test substrate	Artificial soil components Sphagnum peat, air-dried, finely ground 10 % Kaolinite, air-dried 20 % Industrial quartz sand, air-dried 70 % pH = 6.4 Moisture of the test substrate was adjusted to 53.4–56.0 % (w/w) of the maximum water holding capacity (WHC) with deionised water. Total Carbon (TC) and Total Organic Carbon (TOC) content: not stated
Test mixture	Not stated
Size, volume and material of test container	Round glass containers with a diameter of 9.5 cm and a height of 20 cm
Amount of artificial soil (kg)/ container	Wet artificial soil (500 g dry mass) per container
Nominal levels of test concentrations	8.2, 20.5, 51.2, 128.0, 320.0, 800.0, 2000.0, and 5000.0 mg Ampholyt 20 per kg dry soil, corresponding to 1.64, 4.10, 10.24, 25.6, 64.0, 160, 400, and 1000 mg a.s./kg dry soil.
Number of replicates/concentration	4
Number of earthworms/test concentration	40
Number of earthworms/container	10
Light source	Artificial light, 620 lx. The light intensity was measured using an illuminance meter (MINOLTA) with photometric sensor in Lux.
Test performed in closed vessels due to significant volatility of test substrate	No

Table A7.5.1.2- 3: Test conditions.

Criteria	Details
Test temperature	20 °C ± 2 °C
Moisture content	53.4–56.0 % WHC
pH	Please refer to Table A7.5.1.2- 4.
Adjustment of pH	No
Light intensity / photoperiod	Continuous lighting, 620 lx
Relevant degradation products	None

Table A7.5.1.2- 4: Soil pH at test start and test end. Single values of parallel test pots. a.s. = active substance; Concentrations given as nominal concentrations (per kg dry mass)

	Control	1.64 mg a.s./kg	4.10 mg a.s./kg	10.24 mg a.s./kg	25.6 mg a.s./kg	64 mg a.s./kg	160 mg a.s./kg	400 mg a.s./kg	1000 mg a.s./kg
Test start	6.4	6.4	6.5	6.4	6.5	6.5	6.6	6.6	6.8
Test end	7.2	7.2	7.2	7.3	7.2	7.2	7.1	7.1	7.1

Table A7.5.1.2- 5: Mortality data.

Test substance concentration (nominal/measured) [mg/kg artificial soil]	Mortality			
	Number		Percentage	
	7 d	14 d	7 d	14 d
1.64	0	0	0	0
4.10	0	0	0	0
10.24	0	0	0	0
25.6	0	0	0	0
64	0	0	0	0
160	0	0	0	0
400	0	0	0	0
1000	0	0	0	0
Temperature [°C]	20 °C ± 2 °C			
pH	6.4–7.3 (please refer to Table A7.5.1.2-5)			
Moisture content	53.4–56.0 % WHC			

Table A7.5.1.2- 6: Weight change at test end. Weight change compared to the start value [%] and compared to the control [%], a.s. = active substance; Concentrations given as nominal concentrations per kg dry mass. No significant deviation from the control.

Weight change	Control	1.64 mg a.s./kg	4.10 mg a.s./kg	10.24 mg a.s./kg	25.6 mg a.s./kg	64 mg a.s./kg	160 mg a.s./kg	400 mg a.s./kg	1000 mg a.s./kg
Compared to start value [%]	–8.5	–2.6	–4.1	–2.5	–0.1	+2.9	+4.5	–0.3	–3.3
Compared to solvent control [%]		–7.7	–5.0	–6.2	–1.7	–9.9	–5.3	–3.1	–5.4

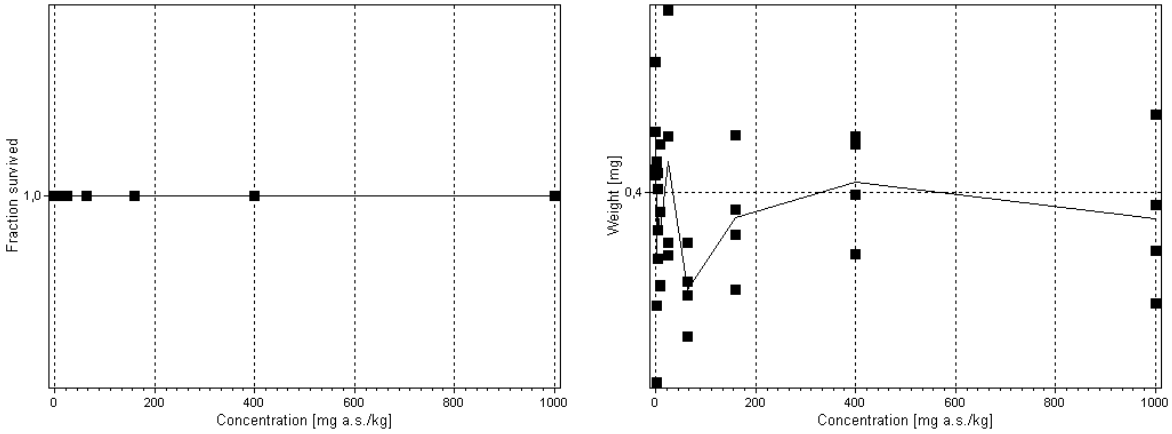

Figure A7.5.1.2- 1: Survival and body weight of *Eisenia fetida* as observed under presence of the test item after 14 d. No concentration/effect curve computable.

Table A7.5.1.2- 7: Effect data.

	14 d [mg/kg soil]	95 % CI
LC_0	≥ 1000	–
LC_{50}	> 1000 (n.d.)	–
LC_{100}	> 1000 (n.d.)	–

Table A7.5.1.2- 8: Validity criteria for acute earthworm test according to OECD 207.

	Fulfilled	Not fulfilled
Mortality of control animals < 10%	Yes	

Section A7.5.1.3 **Terrestrial plant toxicity**
Annex Point IIIA XIII 3.4

Official use only

Reference

Reference	**A7.5.1.3/01:** [redacted] (2007): Terrestrial plants, growth test: Effect of Ampholyt 20 on the seedling emergence and growth of *Avena sativa*, *Lactuca sativa*, *Phaseolus aureus*, *Raphanus sativus*, *Sinapis alba*, and *Triticum aestivum*. Fraunhofer-Institute for Molecular Biology and Applied Ecology (IME), Schmallenberg, Germany. September 17, 2007 (unpublished).
Data protection	Yes
Data owner	Goldschmidt GmbH
Companies with letter of access	No
Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

Guidelines and Quality Assurance

Guideline study	Yes OECD guideline 208 (1984)
GLP	Yes
Deviations	No

MethoD

Test material	As given in Section A2.
Lot/Batch number	ES67345616
Specification	As given in Section A2. The active substance as manufactured is obtained by the production process as a “product-by-process”, constituting a 20 % (w/w) aqueous solution of the active matter.
Purity	20 % (w/w) aqueous solution of pure active matter (99 % w/w)
Composition of Product	20 % a.s. (aqueous solution, “product by process”)
Further relevant properties	The a.s. is a multi-component substance as specified in Section A2. The test substance is hydrolytically stable (water solubility ≥ 200 g/L) and the vapour pressure is 1.9×10^{-4} Pa (20°C) (see section A3).
Method of analysis	None. The test item concentration was not verified by chemical analysis. The test tem is a certified liquid formulation of a known concentration.

Section A7.5.1.3 Terrestrial plant toxicity

Annex Point IIIA XIII 3.4

Preparation of TS solution for poorly soluble or volatile test substances	Not applicable.
Reference substance	Yes, trichloroacetate. A test with the reference substance is regularly performed at the test facility once a year, thus fulfilling guideline recommendations.
Method of analysis for reference substance	Not required according to the test guideline.
Testing procedure	
Dilution water	Source, alkalinity/salinity, hardness, pH, oxygen content and conductance are not explicitly stated.
Test plants	Please refer to Table A7.5.1.3- 1.
Test system	Please refer to Table A7.5.1.3- 2.
Test conditions	Please refer to Table A7.5.1.3- 3.
Test duration	14 days after emergence of 50 % of the control seedlings.
Test parameter	Fresh shoot biomass Pathological symptoms Seedling emergence
Sampling	At growth day 14, all seedlings were counted and the aboveground biomass was measured. For this, the wet mass of the plants was measured immediately after harvesting; see Table A7.5.1.3- 2.
Method of analysis of the plant material	Not applicable, analytical determination of the test substance in plant material not required.
Quality control	Yes
Statistics	The percent inhibition of seedling emergence for each plant species was calculated as an absolute value and in comparison to the control. Survival of emerged seedlings was calculated as an absolute value. The percent inhibition of fresh weight was calculated in comparison to the control. All statistical analyses (Mann-Whitney U-Test, Fisher's Exact Binomial Test, or Welch's t-test for comparison of controls; the Chi^2 2 × 2 Table Test with Bonferroni Correction, Williams' test, or Welch's t-test for the NOEC calculation; EC_{50} estimation, as appropriate) were performed with the computer software ToxRat Professional by ToxRat® Solutions GmbH. For the EC_{50} calculation probit analysis assuming log-normal distribution of the data was applied.

Section A7.5.1.3 Terrestrial plant toxicity

Annex Point IIIA XIII 3.4

Results

Results test substance	
Applied initial concentration	The nominal concentrations in the test containers were 8.2, 20.5, 51.2, 128, 320, 800, 2000, and 5000 mg test item/kg corresponding to 1.64, 4.10, 10.2, 25.6, 64, 160, 400, and 1000 mg a.s./kg dry mass, respectively.
Phytotoxicity rating	No significant pathological symptoms were observed during the test. Please refer to Table A7.5.1.3- 4.
Plant height	Not determined.
Plant dry weights	Not determined but the fresh weights, please refer to Table A7.5.1.3- 7.
Root dry weights	Not applicable.
Root length	Not applicable.
Number of dead plants	Please refer to Table A7.5.1.3- 4.
Effect data	The results of seedling emergence and growth inhibition are presented as mean values in Table A7.5.1.3- 5, Table A7.5.1.3- 6, Table A7.5.1.3- 7, and Table A7.5.1.3- 8.
Concentration / response curve	Please refer to Figure A7.5.1.3- 1.
Other effects	None.
Results of controls	
Number/ percentage of plants showing adverse effects	Emergence was not affected in any tested species: Survival of emerged seedlings in the controls. *Triticum aestivum*: 100% survival *Sinapis alba*: 94% survival *Raphanus sativus*: 100% survival *Phaseoulus aureus*: 95% survival *Lactuca sativa*: 100% survival *Avena sativa*: 100% survival
Nature of adverse effects	Not applicable.
Test with reference substance	
Concentrations	Employed concentrations of trichloroacetic acid are not explicitly reported. However, these are fully documented in report no. IME 002/4-40/2, which is available from the test facility on request.

Section A7.5.1.3 Terrestrial plant toxicity

Annex Point IIIA XIII 3.4

Results

Reference substance: Seedling emergence:

Avena sativa:	Control	97.5 %	100 mg TCA/kg	85.0 %
Lactuca sativa:	Control	85.0 %	100 mg TCA/kg	62.5 %
Phaseoulus aureus:	Control	92.5 %	100 mg TCA/kg	77.5 %
Raphanus sativus:	Control	97.5 %	100 mg TCA/kg	95.0 %
Sinapis alba:	Control	90.0 %	100 mg TCA/kg	80.0 %
Triticum aestivum:	Control	95.0 %	100 mg TCA/kg	90.0 %

EC_{50} values could not be computed.

Reference substance: EC_{50} for growth (fresh mass per plant):

Avena sativa: 6 mg/kg
Lactuca sativa: 55 mg/kg
Phaseoulus aureus: 21 mg/kg
Raphanus sativus: 175 mg/kg
Sinapis alba: 46 mg/kg
Triticum aestivum: 1 mg/kg

Applicant's Summary and conclusion

Materials and methods

The effect of Ampholyt 20 on the emergence and growth of terrestrial plant seedlings was investigated according to the OECD guideline 208. The following test species were used: *Avena sativa* (oat), *Lactuca sativa* (lettuce), *Phaseolus aureus* (mung bean), *Raphanus sativus* (radish), *Sinapis alba* (mustard), and *Triticum aestivum* (wheat).

The seeds were placed in a natural sandy soil containing the test item at nominal concentrations of 1.64, 4.10, 10.2, 25.6, 64, 160, 400, and 1000 mg a.s./kg dry mass, respectively. The concentration of 1000 mg/kg corresponds to the limit test concentration recommended by OECD-guideline 208. The test item was incorporated into the test soil at the beginning of the experiment, and the effects on seedling emergence and growth were determined 14 days after emergence of 50 % of the control seedlings. Four replicates were prepared for the control and per concentration.

Section A7.5.1.3 Terrestrial plant toxicity

Annex Point IIIA XIII 3.4

Results and discussion	There was no concentration dependent effect on seedling emergence of *Avena sativa*, *Phaseolus aureus*, *Raphanus sativus*, and *Triticum aestivum* up to 1000 mg a.s./kg, the highest concentration tested. Thus, the NOEC for these tested species was found to be ≥ 1000 mg a.s./kg dry soil. The EC_{50} was found to be > 1000 mg a.s./kg dry soil. There was a concentration dependent effect on seedling emergence of *Lactuca sativa* and *Sinapis alba* starting at 400 mg a.s./kg (NOEC = 160 mg a.s./kg). However, the EC_{50} for *Lactuca sativa* was found to be 1062 mg a.s./kg (by extrapolation), and that for *Sinapis alba* > 1000 mg a.s./kg dry soil. There were no statistically significant effects on growth (on a fresh weight per plant basis) of *Triticum aestivum* up to the highest test concentration (NOEC ≥ 1000 mg a.s./kg, EC_{50} > 1000 mg a.s./kg). Concentration related inhibition of plant growth was observed in *Avena sativa* (NOEC = 400 mg a.s./kg), *Phaseolus aureus* (NOEC ≤ 1.64 mg a.s./kg), *Raphanus sativus* (NOEC = 10.2 mg a.s./kg), *Lactuca sativa* (NOEC = 160 mg a.s./kg), and *Sinapis alba* (NOEC = 400 mg a.s./kg). However, for the first three of these five species, the effects were only moderate, with the growth rate never being inhibited by more than 50 %. Accordingly, the EC_{50} values for these tested species were found to be > 1000 mg a.s./kg dry soil. For *Lactuca sativa* and *Sinapis alba*, EC_{50} values of 363 mg a.s./kg and 400–1000 mg a.s./kg, respectively, were found.
EC_{20}	–
EC_{50}	EC_{50} values of 363 mg a.s./kg to 1000 mg a.s./kg, depending on the species. NOECs and EC_{50} values for emergence and growth inhibition are given in Table A7.5.1.3- 9 and Table A7.5.1.3- 10, respectively.
EC_{80}	–
Conclusion	According to these results, the test item had a mild inhibitory effect on growth in five of the six tested plant species. With respect to seedling emergence, there was only a mild inhibitory effect in two of the six tested plant species. The validity criteria can be considered as fulfilled (see validity criteria summarized in Table A7.5.1.3- 11).
Reliability	1
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	1st February 2013
Materials and Methods	Pasteurised soil preferable. Concentration of test substance not confirmed by analytical methods.
Results and discussion	Accept applicants version.
Conclusion	Acceptable.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.5.1.3- 1: Test plants.

	Family	Species	Common name	Source
Dicotyledonae				
	Asteraceae	*Lactuca sativa*	Lettuce	Raiffeisen Naturkraft Kiepenkerl
	Fabaceae	*Phaseolus aureus*	Mung bean	Carl Sperling & Co. Lüneburg, Charge 576, D 6210 H
	Brassicaceae	*Raphanus sativus*	Radish	Raiffeisen Naturkraft Kiepenkerl
	Brassicaceae	*Sinapis alba*	White mustard	Landesinstitut für Landwirtschaftliche Qualitätskontrolle
Monocotyledonae				
	Poaceae	*Avena sativa*	Oat	Raiffeisen Genossenschaft Nordwest eG Certification authority Münster - D 993043 D/MS 1160/006
	Poaceae	*Triticum aestivum*	Common wheat	Saatgutveredelung Nueckel, 57392 Schmallenberg-Winkhausen

Table A7.5.1.3- 2: Test system.

Criteria	Details
Test type	Growth chamber test
Container type	Round containers of nonporous plastic with a diameter of 85–95 mm, filled with ca. 280 g of moist soil (natural sandy soil)
Seed germination potential	After emergence of 50 % of the control seedlings, the effects of the test item on seedling emergence and growth were determined. The emergence rate of the control is given in Table A7.5.1.3- 5.
Identification of the plant species	Please refer to Table A7.5.1.3- 1.
Number of replicates	4 replicates per concentration
Numbers of plants per replicate per dose	5 seeds/replicate
Date of planting	Five seeds were planted in each replicate within 24 h after incorporation of the test item
Plant density	Not stated
Date of test substance application	24 h prior to sowing
High of plants at application	Not applicable
Date of phytotoxicity rating or harvest	At growth day 14, all seedlings were counted and the aboveground biomass was measured. For this, the wet mass of the plants was measured immediately after harvesting
Dates of analysis	Not stated

Table A7.5.1.3- 3: Test conditions.

Criteria	Details
Test type	Plant growth chambers
Method of application	Incorporation into soil as an aqueous solution
Application levels	Not applicable (not a plant protection product)
Dose rates	8.2, 20.5, 51.2, 128, 320, 800, 2000, and 5000 mg Ampholyt 20/kg corresponding to 1.64, 4.10, 10.2, 25.6, 64, 160, 400, and 1000 mg a.s./kg dry mass, respectively
Substrate characteristics	The soil used in the test was a natural sandy soil (Certified RefeSol 01-A; batch IME-01, loamy sand [DIN], Org C: 0.93 %, pH 5.7, clay: 5 %). The soil was sieved to 2 mm. The soil was not sterilized. The soil has been stored outdoor in high grade stainless steel basins with drainage and ground contact at the test facility.
Watering of the plants	Continuous bottom watering of the test container via glass fibre wicks. The water was amended with fertilizer (COMBO Grünpflanzendünger)
Temperature	20 ± 2 °C (19–21 °C). The incubation temperature was measured continuously with a thermograph.
Thermoperiod	Not appropriate
Light regime	Illumination period of 16 hours per day with a light intensity of > 7000 lx (light colour 25, universal white).
Relative humidity	60–90 % humidity
Wind volatility	Not applicable
Observation periods and duration of test	14 days after emergence of 50 % of the control seedlings
Pest control	Not appropriate
Any other treatments and procedures	None

Table A7.5.1.3- 4: Pathological symptoms [% plants]; a = discolouration, b = deformation, c = necrosis, d = dead plant.

Test species	Control	1.64 mg a.s./kg	4.10 mg a.s./kg	10.2 mg a.s./kg	25.6 mg a.s./kg	64 mg a.s./kg	160 mg a.s./kg	400 mg a.s./kg	1000 mg a.s./kg
Avena sativa	–	–	–	–	–	–	–	–	–
Lactuca sativa	5 % d	5 % d	-	10 % d	15 % d	–	–	–	5 % d
Phaseolus aureus	–	–	–	–	–	–	10 % d	–	-
Raphanus sativus	–	–	–	–	–	–	–	–	–
Sinapis alba	–	5 % d	10 % d	–	–	–	5 % d	–	–
Triticum aestivum	–	–	–	–	–	–	-	–	–

Table A7.5.1.3- 5: Emergence rate at test end [%]. TI = Test item.

Test species	Control	1.64 mg a.s./kg	4.10 mg a.s./kg	10.2 mg a.s./kg	25.6 mg a.s./kg	64 mg a.s./kg	160 mg a.s./kg	400 mg a.s./kg	1000 mg a.s./kg
Avena sativa	95	100	100	100	85	95	95	90	95
Lactuca sativa	85	80	80	80	90	75	55	55	45
Phaseolus aureus	100	100	100	100	95	100	100	100	100
Raphanus sativus	95	95	100	100	95	100	100	100	100
Sinapis alba	100	85	95	90	95	90	80	75	85
Triticum aestivum	95	75	100	100	95	95	85	100	90

Table A7.5.1.3- 6: Emergence related to solvent control at test end [%]; *: significant when compared with control.

Test species	1.64 mg a.s./kg	4.10 mg a.s./kg	10.2 mg a.s./kg	25.6 mg a.s./kg	64 mg a.s./kg	160 mg a.s./kg	400 mg a.s./kg	1000 mg a.s./kg
Avena sativa	–5	–5	–5	11	0	0	5	0
Lactuca sativa	6	6	6	–6	12	35*	35*	47*
Phaseolus aureus	0	0	0	5	0	0	0	0
Raphanus sativus	0	–5	–5	0	–5	–5	–5	–5
Sinapis alba	15	5	10	5	10	20*	25*	15*
Triticum aestivum	21*	–5	–5	0	0	11	–5	5

Table A7.5.1.3- 7: Fresh weight of the shoots. Mean values ± SD [g] TI = Test item.

Test species	Control	1.64 mg a.s./kg	4.10 mg a.s./kg	10.2 mg a.s./kg	25.6 mg a.s./kg	64 mg a.s./kg	160 mg a.s./kg	400 mg a.s./kg	1000 mg a.s./kg
Avena sativa	0.840	0.933	0.862	0.880	1.073	0.973	0.833	0.815	0.671
Lactuca sativa	1.008	1.277	1.269	1.403	1.227	1.060	1.051	0.405	0.080
Phaseolus aureus	1.699	1.112	0.986	0.959	1.011	0.780	0.936	0.925	0.895
Raphanus sativus	1.727	1.668	1.593	1.628	1.304	1.439	1.601	1.500	0.904
Sinapis alba	0.850	1.046	1.012	1.040	1.247	1.135	1.050	1.223	0.419
Triticum aestivum	0.718	0.776	0.789	0.761	0.761	0.772	0.779	0.719	0.551

Table A7.5.1.3- 8: Growth inhibition related to solvent control at test end [% FM]; TI = Test item; negative value = growth stimulation; *: significant when compared with the control.

Test species	1.64 mg a.s./kg	4.10 mg a.s./kg	10.2 mg a.s./kg	25.6 mg a.s./kg	64 mg a.s./kg	160 mg a.s./kg	400 mg a.s./kg	1000 mg a.s./kg
Avena sativa	–11	–3	–5	–28	–16	1	3	20*
Lactuca sativa	–27	–26	–39	–22	–5	–4	60*	92*
Phaseolus aureus	35	42	44	40	54	45*	46*	47*
Raphanus sativus	3	8	6	25*	17*	7*	13*	48*
Sinapis alba	–23	–19	–22	–47	–34	–24	–44	51*
Triticum aestivum	–8	–10	–6	–6	–8	–9	0	23

Table A7.5.1.3- 9: NOECs and EC_{50} values of Ampholyt 20 for emergence [mg/kg TM]; NOEC = no observed effect concentration; EC = effect concentration; CL = confidence limits; n.d. = not determined for mathematical reasons; nominal concentrations were multiplied with a factor of 0.714 to take application loss into account.

Test species	*Avena sativa*	*Lactuca sativa*	*Phaseolus aureus*	*Raphanus sativus*	*Sinapis alba*	*Triticum aestivum*
NOEC	≥ 1000	64	≥ 1000	≥ 1000	64	≥ 1000
EC_{50} (95% CL)	≥ 1000 (n.d.)	1064 (230–4902)	≥ 1000 (n.d.)	≥ 1000 (n.d.)	≥ 1000 (n.d.)	≥ 1000 (n.d.)

Table A7.5.1.3- 10: NOEC and EC_{50} values for growth inhibition on the basis of fresh mass [mg/kg TM]; NOEC = no observed effect concentration; EC = effect concentration; CL = confidence limits; n.d. = not determined for mathematical reasons; nominal concentrations were multiplied with a factor of 0.714 to take application loss into account.

Test species	*Avena sativa*	*Lactuca sativa*	*Phaseolus aureus*	*Raphanus sativus*	*Sinapis alba*	*Triticum aestivum*
NOEC	400	160	<1.64	10.2	400	≥ 1000
EC_{50} (95% CL)	≥ 1000 (n.d.)	363 (316–394)	≥ 1000 (n.d.)	≥ 1000 (n.d.)	400–1000 (n.d.)	≥ 1000 (n.d.)

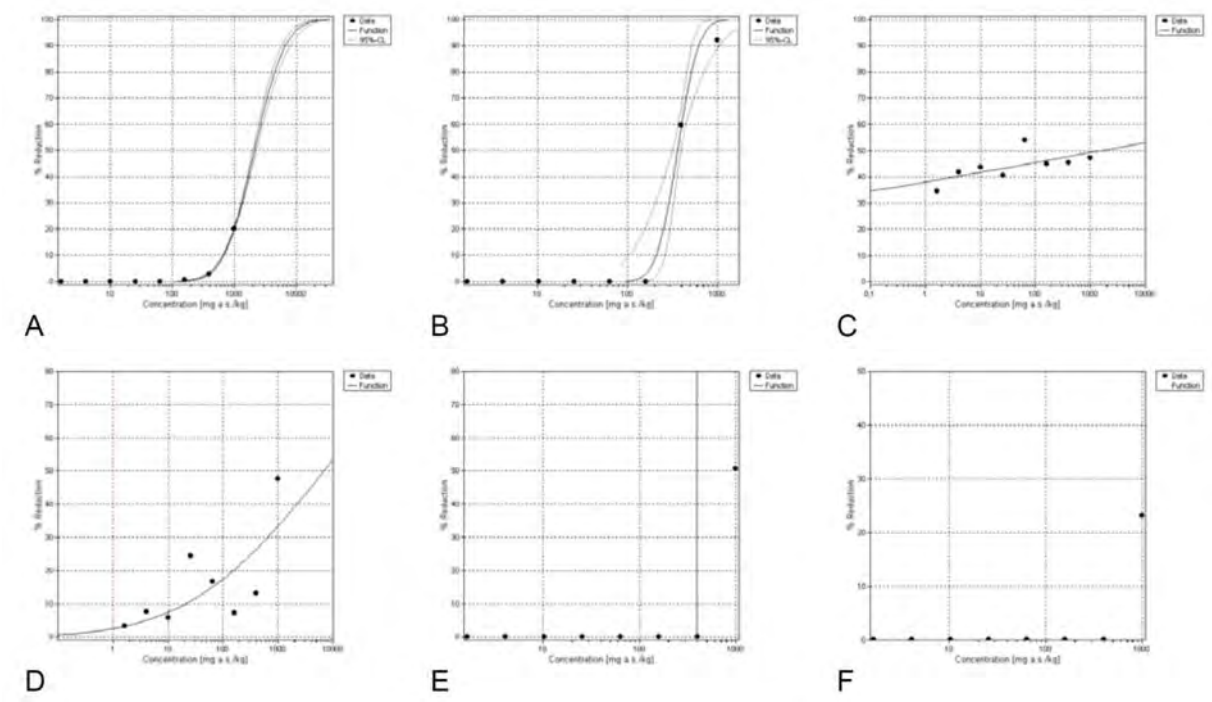

Figure A7.5.1.3- 1: Growth inhibition related to control at test end [% FM]. For computation nominal concentrations were applied. A: *Avena sativa*, B: *Lactuca sativa*; C: *Phaseolus aureus*; D: *Raphanus sativus*; E: *Sinapis alba*; F: *Triticum aestivum*; A, B, C, D: response curve; E, F: no calculation of response curve possible; For C and D confidence limits could not be computed.

Table A7.5.1.3- 11: Validity criteria for terrestrial plant toxicity.

Criterion	Fulfilled	Not fulfilled
The seedling emergence in the controls exceeded 70 % at the end of the test	X	
The control seedlings did not exhibit phytotoxic effects	X	
The mean survival of emerged control seedlings was at least 90 % for the duration of the study	X	
Environmental conditions for a particular species were identical and growing media contained the same amount of soil matrix, support media, or substrate from the same source	X	

Section A7.5.2.1 / Annex Point IIIA 13.3.2 — Terrestrial long-term tests: Reproduction study with other soil non-target macro-organisms

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [X]**	
Detailed justification:	According to chapter 3 of the TNsG on additional data requirements, a test on reproductive effects with soil non-target macro-organisms is required if the risk assessment for the terrestrial compartment, based on the equilibrium partitioning method indicates a concern for the terrestrial compartment or there is long term exposure. However, this is not the case with Ampholyt 20, for the following reasons: (i) The testing for effects on reproductive effects with soil non-target macro-organisms is not considered to be required for lack of exposure, the justification being as follows: The recommended use patterns of Ampholyt 20 are not considered to be involved with any significant direct release to soil. Further, the ready biodegradability and the predicted soil degradation suggest that any long-term exposure to soil organisms should not be expected. Therefore, any quantitatively relevant or long-term exposure of soil non-target macro-organisms is not conceivable. (ii) It is further stated in the TNsG on data requirements that for some product types these tests may be required with the core data set. However, for product types 2–4 (cf. Chapter 2.5) the conduct of these tests is explicitly not required. (iii) The results from the acute toxicity test with soil macro-organisms (Sections A7.5.1.1– A7.5.1.3) do not indicate a significant risk for the terrestrial compartment. Thus, the conduct of a reproduction study with soil macro-organisms is not considered to be required.	
Undertaking of intended data submission [ ]		

Evaluation by Competent Authorities		
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	15th February 2013.	
Evaluation of applicant's justification	Justification accepted.	
Conclusion	Acceptable	
Remarks		
	COMMENTS FROM ...	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A7.5.2.2 Long-term test with terrestrial plants

Annex Point IIIA 13.3.2

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [X]**	
Detailed justification:	According to chapter 3 of the TNsG on additional data requirements, a test for long-term effects on terrestrial plants is required if the risk assessment for the terrestrial compartment, based on the equilibrium partitioning method indicates a concern for the terrestrial compartment or there is long term exposure. (i) Testing of long-term effects on terrestrial plants is not considered to be required for lack of exposure, the justification being as follows: The recommended use pattern of Ampholyt 20 does not involve prolonged or quantitatively relevant release to soil. Further, the ready biodegradability and the predicted soil degradation suggest that any long-term exposure to soil organisms should not be expected. Therefore, any quantitatively relevant or long-term exposure of plants is not conceivable. (ii) It is further stated in the TNsG on data requirements that for some product types these tests may be required with the core data set. However, for product type 2–4, (cf. Chapter 2.5) the conduct of these tests is explicitly not required. (iii) The results from the acute toxicity test with soil macro-organisms (Sections A7.5.1.1– A7.5.1.3) do not indicate a significant risk for the terrestrial compartment. Thus, the conduct of a long-term study with plants is not considered to be required.	
Undertaking of intended data submission [ ]		

Evaluation by Competent Authorities		
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	15th February 2013	
Evaluation of applicant's justification	Justification acceptable	
Conclusion	Acceptable	
Remarks		
	COMMENTS FROM ...	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A7.5.3.1.1 Acute oral toxicity to birds

Annex Point IIIA 13.1.1

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [X]**	
Detailed justification:	From the intended use as a disinfectant, exposure of wild birds to Ampholyt 20 is considered to be extremely unlikely. In view of the very limited exposure, birds are not expected to be at risk. The product in use (a soluble concentrate delivered in plastic containers) is considered to be unattractive to wild birds. It is also noted that this study type is neither a common core data requirement, nor a product-type specific additional data requirement according to the TNsG. Thus, in conclusion, the conduct of an avian acute toxicity study is not considered to be required.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	15th February 2013	
Evaluation of applicant's justification	Justification acceptable	
Conclusion	Acceptable	
Remarks		
	COMMENTS FROM ...	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A7.5.3.1.2 Short-term dietary toxicity to birds

Annex Point IIIA 13.1.2

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [X]**	
Detailed justification:	From the intended use as a disinfectant, exposure of wild birds to Ampholyt 20 is considered to be extremely unlikely. In view of the very limited exposure, birds are not expected to be at risk. It is also noted that this study type is neither a common core data requirement, nor a product-type specific additional data requirement according to the TNsG. Thus, in conclusion, the conduct of an avian acute toxicity study is not considered to be required.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	15th February 2013	
Evaluation of applicant's justification	Justification acceptable	
Conclusion	Acceptable	
Remarks		
	COMMENTS FROM ...	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A7.5.3.1.3 Effects on reproduction in birds

Annex Point IIIA 13.1.3

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [X]**	
Detailed justification:	From the intended use as a disinfectant, exposure of wild birds to Ampholyt 20 is considered to be extremely unlikely. In view of the very limited exposure, birds are not expected to be at risk. It is also noted that this study type is neither a common core data requirement, nor a product-type specific additional data requirement according to the TNsG. Thus, in conclusion, the conduct of an avian acute toxicity study is not considered to be required.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	15th February 2013	
Evaluation of applicant's justification	Justification acceptable.	
Conclusion	Acceptable.	
Remarks		
	COMMENTS FROM ...	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A7.5.4.1 Annex Point IIIA 13.3.1	Acute toxicity to honeybees and other beneficial arthropods	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	The intended use of Ampholyt 20 as a disinfectant is not expected to result in any relevant exposure of bees or other terrestrial arthropods to the active substance. The disinfectant solution is considered to be unattractive to bees. Moreover, the use as a disinfectant, applied onto surfaces in a technical environment (indoors), any residues of the active substance will be unavailable to beneficial insects. Thus, the conduct of any acute honey bee or arthropod toxicity testing is not considered to be required.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	15th February 2013
Evaluation of applicant's justification	Justification Acceptable
Conclusion	Acceptable
Remarks	
	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A7.5.5.1 **Bioconcentration, terrestrial**
Annex Point IIA 7.5

Official use only

Reference

Reference	**A7.5.5.1/01:** [redacted] (2007) Estimation of the terrestrial bioconcentration factor ($BCF_{earthworm}$) of Ampholyt 20. EBRC Consulting GmbH, Hannover, Germany, Report no. DEG-20070704-01, July 03, 2007 (unpublished).
Data protection	Yes
Data owner	Goldschmidt GmbH
Companies with letter of access	No
Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

Guidelines and Quality Assurance

Guideline study	Not applicable. A guideline for the estimation of bioconcentration does not exist. However, estimation was carried out in compliance to the procedure described in the TGD on Risk Assessment, for the estimation of a bioconcentration factor for terrestrial organisms (earthworms). The $BCF_{earthworm}$ was estimated based on the octanol/water partition coefficient (P_{ow}).
GLP	Not applicable.
Deviations	Not applicable.

Materials and Methods

Test material	The test substance Ampholyt 20 is an amphoteric surfactant. It constitutes a complex mixture of partially carboxymethylated alkyl-propylene-diamines, obtained as a “product-by-process”.
Lot/Batch number	Not applicable (theoretical estimation)
Specification	Not applicable (theoretical estimation)
Purity	Not applicable (theoretical estimation)
Further relevant properties	None.
Method of analysis	Not applicable (theoretical estimation)
Reference substance	Not applicable
Method of analysis for reference substance	Not applicable

Section A7.5.5.1 Bioconcentration, terrestrial

Annex Point IIA 7.5

Testing procedure

Test system/ performance	Not applicable (theoretical estimation)
Estimation of bioconcentration	The estimation of the $BCF_{earthworm}$ based on physical-chemical properties (log P_{ow}) as specified by the TGD on risk assessment. The partition coefficient (P_{ow}) of Ampholyt 20 has been calculated on the basis of a model calculation using the established QSAR (quantitative structure activity relationship) software (EpiSuite) for the various individual components of Ampholyt 20 (Horzella 2007, EBRC-No.: GOL-070524-01). Considering the relative proportions of the individual main components, the weighted mean log P_{ow} of Ampholyt 20 is given as 3.81. The original study on the partition coefficient is summarised in Section A3.9/02.

Results

Experimental data

Mortality/ behaviour	Not applicable
Lipid content	Not applicable
Concentrations of test material during test	Not applicable
Bioconcentration factor (BCF)	Not applicable
Uptake and depuration rate constants	Not applicable
Depuration time	Not applicable
Metabolites	Not applicable
Other observations	Not applicable
Estimation of bioconcentration	$BCF_{earthworm} = (0.84 + 0.012 \times P_{ow})/\rho_{earthworm}$ $BCF_{earthworm} = 78.32$

Applicant's Summary and conclusion

Materials and methods	Estimation of the terrestrial bioconcentration factor ($BCF_{earthworm}$) based on the partition coefficient P_{ow}, as specified by the TGD on risk assessment.
Results and discussion	Based on experimentally measured log P_{ow} values (reference A3.9/02) of 3.81, the bioconcentration factor in earthworms was estimated being $BCF_{earthworm}$ = 78.32 l/kg wwt
Conclusion	The bioaccumulation potential of Ampholyt 20 is considered to be low. Since the estimation was performed using an officially recommended model, based on measured values determined under GLP by fully valid experimental procedures, this calculation was considered valid without restrictions.

Section A7.5.5.1 **Bioconcentration, terrestrial**
Annex Point IIA 7.5

Reliability	0 (model calculation)
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	5th March 2013
Materials and Methods	It is not possible to determine the terrestrial BCF for an ionisable substance using this calculation.
Results and discussion	Unacceptable
Conclusion	Unacceptable.
Reliability	
Acceptability	
Remarks	

	Comments from ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A7.5.6 Effects on other terrestrial non-target organisms

Annex Point IIIA 13.3

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	Based on the demonstrated ready biodegradability (Section A7.1.1.2.1) and the low bioconcentration potential (Section A7.4.2) the concern for long-term effects to the terrestrial compartment is considered to be minimal. It is also noted that this study type is neither a common core data requirement, nor a product-type specific additional data requirement according to the TNsG. Thus, the conduct of further studies on terrestrial organisms is not considered to be required.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	20th February 2013	
Evaluation of applicant's justification	This study in not required.	
Conclusion	Acceptable	
Remarks		
	COMMENTS FROM ...	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A7.5.7.1.1 Acute oral toxicity to mammals

Annex Point IIIA 13.3.4

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	Mammalian toxicology is covered in sufficient detail in Section A6. Further risks for mammalian wildlife are not indicated in view of the overall low oral toxicity (Section A6.1.1). In any case, from the intended uses as a disinfectant, exposure of mammals is expected to be negligible. Thus, the conduct of further mammalian toxicity studies is not considered to be required.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	20th February 2013	
Evaluation of applicant's justification	Accept applicants version.	
Conclusion	Acceptable.	
Remarks		
	COMMENTS FROM ...	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A7.5.7.1.2 Short-term toxicity to mammals

Annex Point IIIA 13.3.4

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	Mammalian toxicology is covered in sufficient detail in Section A6. Further risks for mammalian wildlife are not indicated in view of the overall repeated-dose toxicity (Section A6.4.1). From the intended uses as a disinfectant, exposure of mammals is expected to be negligible. Thus, the conduct of further mammalian toxicity studies is not considered to be required.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	20th February 2013	
Evaluation of applicant's justification	Accept applicants version	
Conclusion	Acceptable.	
Remarks		
	COMMENTS FROM ...	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A7.5.7.1.3 Effects on mammalian reproduction

Annex Point IIIA 13.3.4

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	Mammalian toxicology is covered in sufficient detail in Section A6. Further risks for mammalian wildlife are not indicated in view of the general lack of any signs of reproductive toxicity (Sections A6.8.1 and A6.8.2). From the intended uses as a disinfectant, exposure of mammals is expected to be negligible. Thus, the conduct of further mammalian toxicity studies is not considered to be required.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	20th February 2013.
Evaluation of applicant's justification	Accept applicants version.
Conclusion	Acceptable.
Remarks	
	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	