Final Report

Original 2 of 21

Determination of pH-dependent Hydrolysis in Water of
3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctyl acrylate
according to OECD Guideline 111

Study No.: 12100104G916

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1 GLP-Compliance Statement

It is hereby declared that all tests were made in accordance with the „Revised OECD Principles of Good Laboratory Practice" (Paris, 1997) as stated in the following guidelines:

- OECD Principles of Good Laboratory Practice, adopted by Council on 26th November 1997; Environment Directorate, Organisation for Economic Cooperation and Development, Paris 1998
- Directive 2004/10/EC of the European Parliament and of the Council of 11 February 2004 on the harmonisation of laws, regulations and administrative provisions relating to the application of the principles of good laboratory practice and the verification of their applications for tests on chemical substances (codified version)
- Chemikaliengesetz (Chemicals Act) of the Federal Republic of Germany (ChemG) §19a and §19b and annexes 1 and 2 in the version of 02 July 2008 published in Bundesgesetzblatt No. 28/2008, pp. 1146 – 1184, last amended in Federal Law Gazette, Germany (BGBL) from 28. Jan. 2013, N. 03/2013, S. 94.

Responsibility for the accuracy of the information concerning the test item as well as for its authenticity rests with the sponsor.
I herewith accept responsibility for the data presented within this report.
There were no circumstances that may have affected the quality or integrity of the study.

This report contains the following data which was not acquired under GLP conditions:
GC/MS analysis of the extract (pH 9), performed at the laboratory Institut Dr. Appelt in Mannheim, Germany.

28 MAR 2013

Date

Study Director

Information on Study Organisation:

Deputy Study Director

Study Plan dated	19. Nov. 2012
Experimental Starting Date	21. Nov. 2012
Experimental Completion Date	14. Mar. 2013
Draft Report dated	27. Mar. 2013

2 QUALITY ASSURANCE UNIT STATEMENT

This study has been inspected by the quality assurance unit according to the principles of Good Laboratory Practice. Study Plan and Final Report were checked at the dates given below, the Study Director and the management were informed with the corresponding report.
Also, the performance of the study was inspected, and findings were reported to Study Director and management. The inspection of short-term studies (duration less than four weeks) is carried out as audit of process concerning major technical phases of at least one similar test. Frequency is once or more a quarter.
The study was conducted and the reports were written in accordance with the Study Plan and the Standard Operating Procedures of the test facility.
Deviations from the Study Plan were acknowledged and assessed by the Study Director and included in the Final Report.
The reported results reflect the raw data of the study.

Verified Procedure	Inspected on	Findings reported on	Audit report no.
Study plan	14. Nov. 2012	14. Nov. 2012	121114-04
Performance of study	26. Nov. 2012	26. Nov. 2012	121126-01
Draft report	28. Feb. 2013 27. Mar. 2013	01. Mar. 2013 27. Mar. 2013	130228-08 130327-02
Final report	28. Feb. 2013	28. Feb. 2013	130328-02

28 MAR 2013
Date

Quality Assurance Manager

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3 SUMMARY

Title of Study: Determination of pH-dependant Hydrolysis in Water of 3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctyl acrylate according to OECD Guideline 111

Findings and Results:

Tier 1

A solution of the test item in sterilised water was mixed with sterilised buffer solutions (pH values: 4; 7; and 9). The resulting solutions were stored at 50 °C for a period of five days. Samples were taken at the beginning and after five days. The analysis of the samples (performed with GC/FID) showed significant changes in the concentration of the test item within five days.

Table 3-a Results Tier 1

pH	% of start concentration after 5 days	% decrease within 5 days
4	13 %	87 %
7	17 %	83 %
9	< 1 %	> 99 %

On the base of these results, Tier 2 was performed at all pH values.

Tier 2

A solution of the test item in sterilised water was mixed with sterilised buffer solutions (pH values: 4; 7; and 9). The resulting solutions were stored at 10, 25, and 50 °C. Sampling was performed in suitable time intervals in order to monitor the hydrolysis behaviour of the test item at the different temperatures and pHs.
Analysis of the samples was performed with GC/FID.
The following hydrolysis constants and half-lives were determined at the three pH values and the three temperatures (see following page):

Table 3-b Results Tier 2

Temperature [°C]	pH	K_{obs} [h^{-1}] (pH)	Half-life [h]	K_{obs} [h^{-1}] (total)	Half-life [h] (total)
50	4.00	0.027544	25.2	0.116322	5.96
	7.00	0.021094	32.9		
	9.00	0.067684	10.2		
25	4.00	0.018586	37.3	0.052312	13.25
	7.00	0.018271	37.9		
	9.00	0.015455	44.8		
10	4.00	0.014682	47.2	0.039416	17.59
	7.00	0.013679	50.7		
	9.00	0.011056	62.7		

K_{obs} (total) was calculated as sum of the experimentally determined k-values. Half-life was calculated from ln(2)/k.

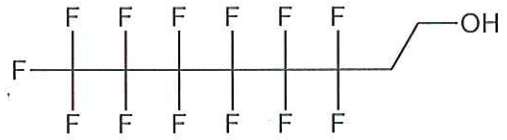
Using the Arrhenius equation, the following parameters were calculated for hydrolytical behaviour of 3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctyl acrylate at 20 °C:

K_{obs} = 0.04917
$t_{1/2}$ = 14.1 h

Tier 3

A solution of the test item in sterilised water was mixed with sterilised buffer solution (pH 9). The resulting solution was stored at 50 °C. Sampling was performed after 42.5 hours and the extract was measured via GC-MS.

One additional signal was observed in the GC-chromatogram (retention time approx. 2.6 min.). Following GC/MS-Analysis, this signal is the expectable hydrolysis product 3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctyl alcohol:



FC(F)(C(F)(F)CCO)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F

4 PURPOSE AND PRINCIPLE OF THE STUDY

This study was performed in order to determine the hydrolysis behaviour of 3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctyl acrylate in dependence of the pH. Sterile aqueous buffer solutions of different pH values (pH 4, 7 and 9) were treated with the test item and incubated in the dark under controlled laboratory conditions (at constant temperatures). After appropriate time intervals, buffer solutions were analysed for the test item. The main hydrolysis product was identified.

Sponsor's intent: registration in accordance with: REACH.

5 LITERATURE

The study was conducted in accordance with the following guidelines:

- OECD-Method 111: „Hydrolysis as a fiunction of pH", 13. Apr. 2004

Corresponding SOP of LAUS GmbH:

- •SOP 118 009 16 "Bestimmung der Hydrolyse als Funktion des pH", edition 4 valid from 01. Sep. 2011

6 MATERIAL AND METHODS

6.1 Test Item

Designation in Test Facility:	12100104G
Date of Receipt:	01. Oct. 2012
Condition at Receipt	room temperature, in proper conditions

6.1.1 Specification

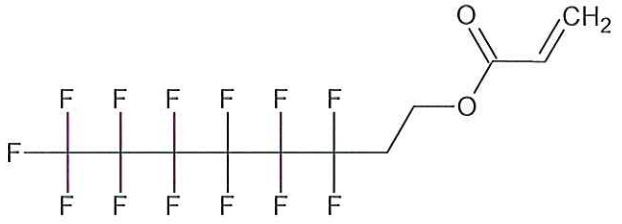
The following information concerning identity and composition of the test item was provided by the sponsor.

Name	3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctyl acrylate
Batch	6SFCC96119
Appearance	colourless liquid
Composition	90 % 2-Propenoic acid, 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl ester, 7% water, 3% acrylic acid
CAS-No.	17527-29-6
EINECS-No.	241-527-8
Molecular formula	not stated
Molecular weight	not stated
Purity	99.5% (GC)
Homogeneity	not stated
Solubility	not stated
Production date	Jun. 2009
Expiry date	24. Sep.2013
Storage	Room Temperature: (20 ± 5°C)
Stability	not stated
Hazard information	Xi irritant
R-phrases	R36/37/38: Irritating to eyes, respiratory system and skin.
S-phrases	S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S37: Wear suitable gloves.

6.1.2 Storage

The test item was stored in a tightly closed vessel at room temperature, in a dry well ventilated place.

6.1.3 Structure and SMILES Code



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6.2 Test System

6.2.1 Glassware

All glassware was autoclaved before use. Duran 3.3 was used. Glass flasks, nominal volume 100 mL, with teflon seals were used as test flasks.

6.2.2 Incubation Chambers

Climate chambers, LAUS no. 6 (Tier 1) and LAUS no. 14 (Memmert) (Tier 2), adjustable to 10 °C, 25 °C and 50 ± 0.1 °C. Climate chamber (OxiTop-chamber), LAUS no. 3 (Tier 2, pH 4, 25 °C, repetition) was used for a short time only. Usage and calibration following the corresponding SOPs in the current edition.

6.2.3 pH-Meter

wtw pH 540 GLP. Usage and calibration following the corresponding SOP in the current edition.

6.2.4 Thermometer

Glass thermometer LAUS Nos.: 20110207_104, 20020912_25

6.3 Other Instruments and Devices

The following instruments and devices were used for the performance of the study:

- Autoclav Sanyo MLS 3020
- Membrane filters 0.2 µm
- Syringes 5 mL
- Precision scales Mettler Toledo XS6001S
- Analytical scales Mettler Toledo XS205DU LAUS No. 2
- Adjustable pipettes with one-way tips, LAUS No.: 14, 30, 43, 44, 45, 48, 64
- Gas chromatograph GC 6890 Agilent

Usage and calibration following the corresponding SOP in the current edition.
Standard laboratory material (e.g. glassware) was also used in the performance of the study

6.4 Chemicals and Reagents

6.4.1 Water

Deionised water was used for the solution of the test item and for the preparation of the buffer solutions. Deionised water for the test item solution was previously autoclaved.

6.4.2 Test Item Solution

All solutions were autoclaved or sterile filtrated before addition of the test item, as the the test item absorbs to membrane filters and no filtration step can be used after addition of the test item to the solvent!

The preparation of the test item solution for Tier 1 and Tier 2 is described in chapter 7.

6.4.3 Buffer Solutions

Composition was taken from the annex of the OECD method (buffer solutions 7 and 9) resp. Küster et al. (buffer solution 4). All chemicals used were of analytical grade. The pH was measured with a pH-meter with an uncertainty of 0.01 units and pH was adjusted to the nominal pH value ± 0.02 units. The buffer solutions were autoclaved, resulting in sterile solutions.

6.4.3.1 Acetic acid, 2-m
CH_3COOH, p.A., concentration 2 mol/L

6.4.3.2 Sodium acetate solution, 1-m
CH_3COONa, p.A., concentration 1 mol/L

6.4.3.3 Buffer-Solution, pH 4

CH_3COOH, 2-m	160.0 mL
CH_3COONa, 1-m	80 mL
Water	ad 2000 mL

6.4.3.4 Potassium dihydrogenphosphate
KH_2PO_4, p.A.

6.4.3.5 Sodium hydroxide solution, 0.1-m
NaOH, p.A., 0.1 mol/L

6.4.3.6 Buffer-Solution, pH 7

KH_2PO_4 (Tier 1)	17.4171 g
KH_2PO_4 (Tier 2, 25 °C, 10 °C)	17.4169 g
KH_2PO_4 (Tier 2, 50 °C)	17.4170 g
Water	500 mL
NaOH, 2-m	29.8 mL
Water	ad 2000 mL

6.4.3.7 Boric acid
H_3BO_3, p.A.

6.4.3.8 Potassium chloride
KCl, p.A.

6.4.3.9 Buffer-Solution, pH 9

H_3BO_3 (Tier 1)	6.1863 g
H_3BO_3 (Tiers 2 and 3)	6.1862 g
KCl (Tier 1, Tier 2, 25 °C)	7.4579 g
KCl (Tier 2, 10 °C)	7.4578 g
KCl (Tiers 2 and 3, 50 °C)	7.4582 g
Water	1000 ml
NaOH, 2-m	21.5 mL
Water	ad 2000 mL

6.4.4 Ar, free from O_2
Argon, free of oxygen, was used.

6.5 Analytical Method

6.5.1 Gas Chromatograph (GC3) with FID

hp-numbers:	GC-Model 6890N (G1530N), Serial number US10241004 Auto sampler G1513A, Serial number CN23721839 Auto injector 18596C, Serial number CN23927412 Computer Vectra VL 420 DT, serial number FR23416281 Printer hp LaserJet 2200, Serial number CNKS B03 972
manufacturer:	Hewlett Packard (= hp), new „AGILENT Technologies", at
date of purchase:	Nov. 2002
Special software:	ChemStation Rev. A.09.03 [1417] *ChemStation Plus* ChemStore C/S Rev. B.02.01, Security-Pack and Chem Access Software *ChemStation Plus* ChemStore C/S SR2/3 for Rev. B.02.01 *ChemStation Plus* User Documentation Rev. A.09.01 GC ALS Upgrade Disk Rev. 08/01
General software:	"Recovery CD-ROM for HP Vectra VL 420 P621xW/P6825W Windows 2000 SP2", disks 1 and 2 drivers and utilities cd-rom ("hp pc image engineer – image library and diagnostics") for use with hp vectra vl420 cd writer software "HP RecordNow HP DLA" for windows 2000 (u.a.) "hp jetdirect cd-rom" for Windows 2000 (u.a.) "hp usb internet keyboard cd-rom" for Windows 2000 (u.a.) 10/100 PCI Network Interface Cards Installation CD "EtherCD CD-ROM Version 5.4" for Windows 2000 (u.a.)

Usage following the corresponding SOP 11400507 in the current edition (GC3).

6.5.2 Parameters of Instrument GC3

Column	Rtx-440, 30 m * 0.25 mm * 0.25 µm
Temperature	50 °C/1 min. isothermal, 20 °C/min. to 300 °C
Gas Type	H_2
Inlet	280 °C, splitless
Detector	FID, 300 °C

6.5.2.1 Method Characterisation

Linear sector of method:	1 – 80 mg/L
Limit of quantification:	0.1 mg/L (lowest level of the calibration 1 mg/L taking into account the tenfold concentration of the sample solution)
Limit of detection:	0.1 mg/L

Recovery rates from the buffer solutions:

pH 4 buffer solution:	93 %
pH 7 buffer solution	92 %
pH 9 buffer solution	89 %

6.5.3 Parameters of Instrument GC/MS (Tier 3)

Parameters of Instrument GC/MS see chapter 15, page 47.

6.5.4 Measurements Tiers 1 and 2

6.5.4.1 System Stability (Tier 1)

A QC sample (50 mg/L) was measured in tier 1 on day 0 and on day 5. The area of the standard was compared on days 0 and 5 resulting in a recovery rate of 98 %. Calculation of the test item concentrations in the test solutions was not necessary; hydrolytical behaviour could be assessed on the base of measured areas.

6.5.4.2 Calibration Data of 24. Jan. 2013 (Tier 2)

The data is presented in the following table:

Table 6.5-a Measured Areas

Conc. [mg/L]	Area 1 Test Item [pA*s]	Area 2: Test Item [pA*s]	Area Mean: Test Item [pA*s]	Area Standard deviation [pA*s]
1	11.04	10.98	11.01	0.04245
5	30.51	28.51	29.51	1.40972
10	67.48	70.11	68.80	1.85707
30	153.11	159.39	156.25	4.44186
50	263.30	265.98	264.64	1.89454
80	421.20	412.27	416.73	6.31716

The parameters of the calibration function are given in the following table:

Table 6.5-b Calibration Parameters Linear Calibration

Slope	5.107302098	pA*s / mg/L
Intersection y-axis	8.010017999	pA*s
Residual standard deviation	5.947512625	pA*s
Method standard deviation	1.164511617	mg/L
Method variation coefficient	3.97	%
Correlation coefficient r	0.9994311	
Coefficient of determination r^2	0.998862524	

The concentration of the test item in buffer solutions (tier 2, 10 °C and 25 °C) was determined using the following equation:

Concentration (mg/L) = (Area – 8.010017999) / 5.107302098

6.5.4.3 Recovery Rate of Quality Control Samples
Quality control samples were measured on the sampling days without calibration. The results are given in the following table:

Table 6.5-c Recovery Rate of QC Samples

Temperature	Day	Recovery in %
25 °C	1	91 %
	2	93 %
	4	91 %
	5	93 %
25 °C, pH 4 (Repetition)	0	93 %
	1	91 %
	2	90 %
	3	94 %
10 °C	5	93 %
	7	93 %

6.5.4.4 Calibration Data on 18. Feb. 2013 (Tier 2, 50 °C)
As the recovery rate of the QC sample was 88 % on day 0 (50 °C) new calibration was performed. The data is presented in the following table:

Table 6.5-d Measured Areas

Conc. [mg/L]	Area 1 Test Item [pA*s]	Area 2: Test Item [pA*s]	Area Mean: Test Item [pA*s]	Area Standard deviation [pA*s]
1	9.07	9.48	9.27	0.29418
5	26.02	25.01	25.52	0.71187
*10**	*58.41*	*54.17*	*56.29*	*2.99574*
30	127.49	130.95	129.22	2.45140
50	211.96	219.25	215.60	5.14858
80	364.52	372.28	368.40	5.48634

***Level 3 (10 mg/L) showed great deviation and was not used in the calculation of the calibration function**

Both (linear and quadratic) calibration functions were calculated.

Values for linear function y = bx + a

Table 6.5-e Calibration Parameters Linear Calibration

Slope	4.503459124	1 / mg/L
Intersection y-axis	0.087775481	1 / mg/L
Residual standard deviation	8.637894086	
Method standard deviation	1.918057619	mg/L
Method variation coefficient	5.78	%
Correlation coefficient r	0.998723497	
Coefficient of determination r^2	0.997448624	

Values for quadratic function $y = cx^2 bx + a$

Table 6.5-f Calibration Parameters Quadratic Calibration

c	0.009774955	1 / $(mg/L)^2$
b	3.737957897	1 / mg/L
a	6.292674484	
Residual standard deviation	2.120224386	mg/L
Method standard deviation	0.483295459	mg/L
Method variation coefficient	1.46	%
Coefficient of determination r^2	0.9999	

As the quadratic model fits much better, the quadratic function was used.

The concentration of the test item in buffer solutions (tier 2, 50 °C) was determined using the following equation:

$$Conc.[mg/L] = \frac{b}{2*c} + \sqrt{\left(\frac{b}{2*c}\right)^2 - \left(\frac{a - Area}{c}\right)}$$

6.5.5 Measurements (Tier 3)
See chapter 15, page 47.

6.5.6 Sample Preparation Tiers 1 and 2

To 100 mL sample, 5 g NaCl was added; then, the solution was extracted two times with the solvent methyl t-butyl ether (9 mL, 4 mL), the organic phase was collected after drying with Na_2SO_4 into a 10 mL flask and the flask was filled up to 10 mL with methyl t-butyl ether. Tenfold enrichment was achieved.

6.5.7 Sample Preparation Tier 3

Two replicates were extracted. To 100 mL sample, 5 g NaCl was added each; then, the solutions were extracted two times with the solvent methyl t-butyl ether (9 mL, 4 mL), the organic phases from both replicates were collected after drying with Na_2SO_4 into a 25 mL flask and the solution was sent to the laboratory Dr. Appelt for GC/MS analysis.

7 PERFORMANCE OF THE STUDY

All solutions and glass ware were sterilised before addition of the test item.

For tier 1, a solution of the test item was prepared by spiking of 400 mL autoclaved demineralised water with 4 mL of test item solution in methanol (1000 mg/L), resulting in a nominal concentration of the test item in water of 10 mg/L. This sterile solution was mixed 1:1 with the appropriate sterile buffer solution, giving a concentration of 5 mg/L.

Three test flasks (100 mL) for each pH buffer were filled with the respective test solution leaving no headspace. The flasks were closed using teflon seals. One blank for each pH buffer were prepared. All flasks were stored at 50 °C in an incubation chamber. The residuals of the test solutions were extracted immediately, giving the initial values.

For tier 2 (10 °C and 25 °C, all pH values), a solution of the test item was prepared by spiking of 1000 mL demineralised water with 6 mL of test item solution in methanol (2000 mg/L), resulting in a nominal concentration of the test item in water 12 mg/L. This sterile solution was mixed 1:1 with the appropriate sterile buffer solution (1200 mL buffer + 200 mL water). 16 test flasks (100 mL) for each pH buffer were filled with the test solution leaving no headspace. The flasks were closed using teflon seals. All flasks were stored at the test temperature in an incubation chamber. The residual of the test solution were extracted immediately, giving an initial value.

For tier 2 (pH 4 at 50 °C), a solution of the test item was prepared by spiking of 1493 g demineralised water with 7.5 mL of test item solution in methanol (2000 mg/L), resulting in a nominal concentration of the test item in water approx. 10 mg/L. This sterile solution was mixed 1:1 with the appropriate sterile buffer solution (1500 mL) and 20 test flasks (100 mL) were filled with the test solution leaving no headspace.

For tier 2 (pHs 7 and 9 at 50 °C), a solution of the test item was prepared by spiking of 1300 g demineralised water with 6.5 mL of test item solution in methanol (2000 mg/L), resulting in a nominal concentration of the test item in water approx. 10 mg/L. This sterile solution was mixed 1:1 with the appropriate sterile buffer solution (1300 mL) and 16 test flasks (100 mL) for each pH buffer were filled with the test solution leaving no headspace.

For each sampling time, two fresh flasks were used to avoid microbial contamination and contact with oxygen.

For tier 3 (pH 9), a solution of the test item was prepared by spiking of 199 g demineralised water with 1 mL of test item solution in methanol (2000 mg/L), resulting in a nominal concentration of the test item in water approx. 10 mg/L. This sterile solution was mixed 1:1 with the sterile buffer solution (200 g), two test flasks (100 mL) were filled with the test solution leaving no headspace and stored at 50 °C for approximately 42.5 hours in order to obtain nearly complete hydrolysis of the test item.

7.1 Tier 1

Performance:	21. – 26. Nov. 2012
pH values:	4.0; 7.0; 9.0
Buffers:	see chapter 6.4.3
Test temperature:	50 ± 0.5 °C
Number of flasks:	3 per pH

7.2 Tier 2

7.2.1 Test at 50 °C

Performance:	18. – 22. Feb. 2013
pH value:	4, 7 and 9
Buffers:	see chapter 6.4.3
Test temperature:	50.0 °C
Number of flasks:	16 each for pH 7 and pH 9, 20 for pH 4

7.2.2 Test at 25 °C

Performance:	24. – 29. Jan. 2013 11 – 14. Feb. 2013 (repetition pH 4)
pH value:	4, 7 and 9
Buffers:	see chapter 6.4.3
Test temperature:	25.0 °C
Number of flasks:	16 per pH

7.2.3 Test at 10 °C

Performance:	04. – 11. Feb. 2013
pH value:	4, 7 and 9
Buffers:	see chapter 6.4.3
Test temperature:	10.0 °C
Number of flasks:	16 per pH

7.3 Tier 3

Performance:	11. – 14. Mar. 2013
pH value:	9.0
Buffer:	see chapter 6.4.3
Test temperature:	50.0 °C
Number of flasks:	2

8 CALCULATION OF RESULTS

8.1 Analytical Values

Concentrations were calculated as follows

$$c_m = \frac{\text{Area} - \text{Intercept}}{\text{slope}}$$

with

c_m = measured concentration
Area = measured area by GC/FID

or

$$c_m[mg/L] = \frac{b}{2*c} + \sqrt{\left(\frac{b}{2*c}\right)^2 - \left(\frac{a - Area}{c}\right)}$$

Slope, intercept and a, b, c are the coefficients of the corresponding calibration function (see chapters 6.5.4.2 and 6.5.4.4).

All measured concentrations c_m were multiplied by the reciprocal value of enrichment factor, which is always 0.1, recovery rate of the test item from the buffer solutions and, if applicable, the correction factor, calculated from the measured QC samples.

$$c = \frac{c_m * 0.1 * 100\%}{RR(TI) * RR(QC)}$$

with

c = corrected concentration
RR(TI) = recovery rate of the test item from the respective buffer solution (93 % pH 4, 92 % pH 7 and 89 % pH 9)
RR(QC) = recovery rate of QC sample

8.2 Hydrolysis

Hydrolysis in % was calculated from the following equation:

$$H = \frac{c_t}{c_0} * 100\%$$

with

c_t = corrected concentration at time t
c_0 = corrected concentration at time 0

8.3 Kinetic Parameters

Hydrolysis refers to a reaction of a substance RX with water:

$RX + H_2O \rightarrow ROH + RX$

Since H_2O is present in great excess, kinetic usually can be considered as being first order. The corresponding equation for the rate of hydrolysis is calculated from:

$rate = k * [RX]$

The hydrolysis constant K_{obs} (k observed) at a defined pH and temperature can be determined from:

$k_{obs} = k * [H_2O]$

and

$$k_{obs} = \frac{1}{t} * \ln\frac{C_0}{C_t} = \frac{2{,}303}{t} * \log\frac{C_0}{C_t}$$

The half-life of the compound at a defined pH and temperature can then be calculated from:

$$t_{0,5} = \frac{\ln 2}{k_{obs}}$$

For the calculation of hydrolysis as a function of temperature, the following equation applies:

$$k_{obs} = k_H[H^+] + k_{neutr.} + k_{OH}[OH^-] = \sum_{I=H,neutr.,OH} A_i e^{-B_i / T}$$

with

k_H	hydrolysis constant at pH 4
k_{neutr}	hydrolysis constant at pH 7
k_{OH}	hydrolysis constant at pH 9
A_i	respective intercept
B_i	respective slope
T	temperature in K

Slope and intercept for each pH are generated from linear regression of ln k_i against 1/T (this regression yields the intercept ln A and the slope – B).

9 FINDINGS AND RESULTS

For all measured values applies:

c_m is the value calculated from area and the respective calibration function.

c includes the recovery rate of the standards and the enrichment factor of 0.1.

9.1 Tier 1

9.1.1 Measured Concentrations, Decrease

The measured areas before and after the storage of the vessels at 50 °C for 5 days and the decrease are presented in the following table:

Table 9.1-a Measured Concentrations Tier 1

Parameter	**pH 4.0**	**pH 7.0**	**pH 9.0**
Area Mean 0 h	236.83	196.51	224.60
Area blank	< LOD	< LOD	< LOD
Area 120 h Mean	29.60	33.89	< 1
Area 120 h blank	not measured	< LOD	< LOD
Residue	**13 %**	**17 %**	**< 1 %**
Decrease	87 %	83 %	> 99 %

9.1.1 Assessment

After five days (120 hours), the areas of the test item were lower than 17 % of the start area at all three pH values. The test item can be considered as hydrolytically instable at all three pH values. Following the guidelines, tier 2 has to be conducted.

9.2 Tier 2

The tests at the different temperatures and pH values are reported individually. Two replicates were extracted at each sampling point, each replicate was measured twice.

9.2.1 Test at 10 °C

9.2.1.1 pH 4.00

The values which were measured at 10 °C are presented in the following table:

Table 9.2-a Areas pH 4, 10 °C

Sampling Time [h]	Replicate 1		Replicate 2	
	Area 1 [pA*s]	Area 2 [pA*s]	Area 1 [pA*s]	Area 2 [pA*s]
0.00	247.06	248.61	234.12	233.88
22.50	206.74	218.37	233.52	229.55
46.50	171.58	169.99	177.92	174.91
53.50	152.26	158.00	146.21	144.01
70.67	118.42	118.88	135.80	124.71
77.50	129.18	124.28	105.03	109.72
94.17	86.26	89.66	83.50	86.57
100.92	70.62	72.59	84.56	83.59
119.25	56.16	56.60	60.46	56.85

The concentration of the test item at each sampling time was determined using equation which is described in chapters 6.5.4.2 and 8.1 taking into account the recovery rate of the test item (93 %). Additionally, on day 5 (119.25 h), the recovery rate of the QC sample (93 %) was taken into account.

Table 9.2-b Concentration (c_m) at pH 4 and 10 °C

Sampling Time	Factor	Replicate 1		Replicate 2		Conc. Mean
[h]		Conc. 1 [mg/L]	Conc. 2 [mg/L]	Conc. 1 [mg/L]	Conc. 2 [mg/L]	[mg/L]
0.00	0.1	5.03	5.07	4.76	4.76	4.91
22.50	0.1	4.18	4.43	4.75	4.66	4.51
46.50	0.1	3.44	3.41	3.58	3.51	3.49
53.50	0.1	3.04	3.16	2.91	2.86	2.99
70.67	0.1	2.32	2.33	2.69	2.46	2.45
77.50	0.1	2.55	2.45	2.04	2.14	2.30
94.17	0.1	1.65	1.72	1.59	1.65	1.65
100.92	0.1	1.32	1.36	1.61	1.59	1.47
119.25	0.1	1.09	1.10	1.18	1.10	1.12

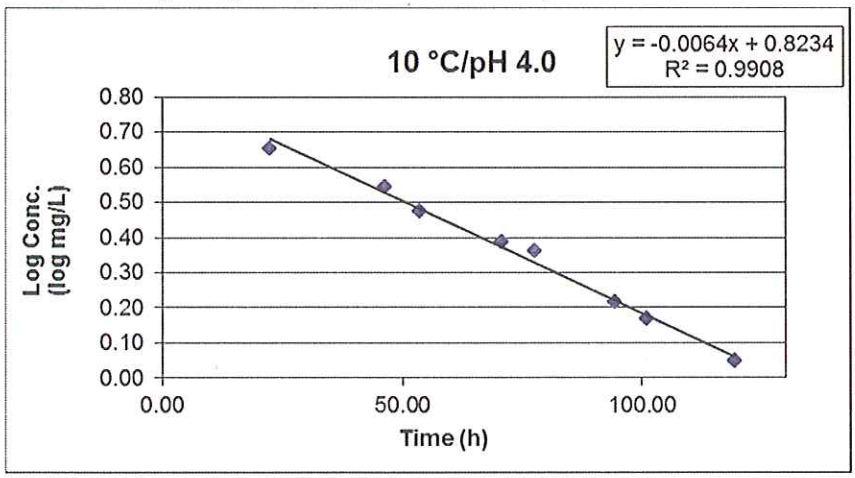
From the measured concentrations, the following percentage hydrolysis was calculated:

Table 9.2-c Hydrolysis pH 4, 10 °C

Sampling Time [h]	Recovery [%]	Decrease [%]	log C [log mg/L]
0.00	*100.0*	*0.0*	*0.6911*
22.50	91.9	-8.1	0.6542
46.50	71.1	-28.9	0.5428
53.50	60.9	-39.1	0.4757
70.67	49.9	-50.1	0.3892
77.50	46.8	-53.2	0.3617
94.17	33.6	-66.4	0.2175
100.92	29.9	-70.1	0.1673
119.25	22.8	-77.2	0.0492

The value, which is printed in *grey italics (0 h),* showed great deviation from the linear regression and was not used in the calculation of the hydrolysis function.

Graph of Hydrolysis (range 92 – 23 %):



Kinetic parameters were calculated from the regression linear using the equations given in chapter 8.3 (k_{obs} = (-2.303)*slope) and are presented in the following table:

Table 9.2-d Kinetic Parameters pH 4, 10 °C

Parameter	Value	Unit
Slope	-0.006375	log mg/L / h
k_{obs} (10 °C; pH 4.0)	0.014682	h^{-1}
$t_{1/2}$ (10 °C; pH 4.0)	47.2	h

9.2.1.2 pH 7.00
The values which were measured at 10 °C are presented in the following table:

Table 9.2-e Areas pH 7, 10 °C

Sampling Time [h]	Replicate 1		Replicate 2	
	Area 1 [pA*s]	Area 2 [pA*s]	Area 1 [pA*s]	Area 2 [pA*s]
0.00	237.67	238.78	232.02	231.18
21.58	220.12	212.62	214.96	218.71
45.58	167.61	164.55	163.64	148.91
52.75	163.65	163.71	156.94	153.43
69.92	119.15	120.21	128.97	132.05
76.75	107.59	*95.68**	107.20	116.08
93.50	86.27	92.63	88.73	86.32
100.17	78.77	86.96	76.03	74.25
118.50	69.71	62.06	56.87	-**

***The value was not used in the calculation of the mean due to measuring error**
****Due to an instrumental failure, the chromatogram was not available**

The concentration of the test item at each sampling time was determined using the equation which is described in chapters 6.5.4.2 and 8.1 taking into account the recovery rate of the test item (92 %). Additionally, on day 5 (118.5 h), the recovery rate of the respective QC sample (93 %) was taken into account.

Table 9.2-f Concentration (c_m) at pH 7 and 10 °C

Sampling Time	Factor	Replicate 1		Replicate 2		Conc. Mean
[h]		Conc. 1 [mg/L]	Conc. 2 [mg/L]	Conc. 1 [mg/L]	Conc. 2 [mg/L]	[mg/L]
0.00	0.1	4.89	4.91	4.77	4.75	4.83
21.58	0.1	4.51	4.35	4.40	4.48	4.44
45.58	0.1	3.40	3.33	3.31	3.30	3.35
52.75	0.1	3.31	3.31	3.17	3.09	3.22
69.92	0.1	2.37	2.39	2.57	2.64	2.49
76.75	0.1	2.12	*1.87*	2.11	2.30	2.18
93.50	0.1	1.67	1.80	1.72	1.67	1.72
100.17	0.1	1.51	1.68	1.45	1.41	1.51
118.50	0.1	1.41	1.23	1.11	-	1.25

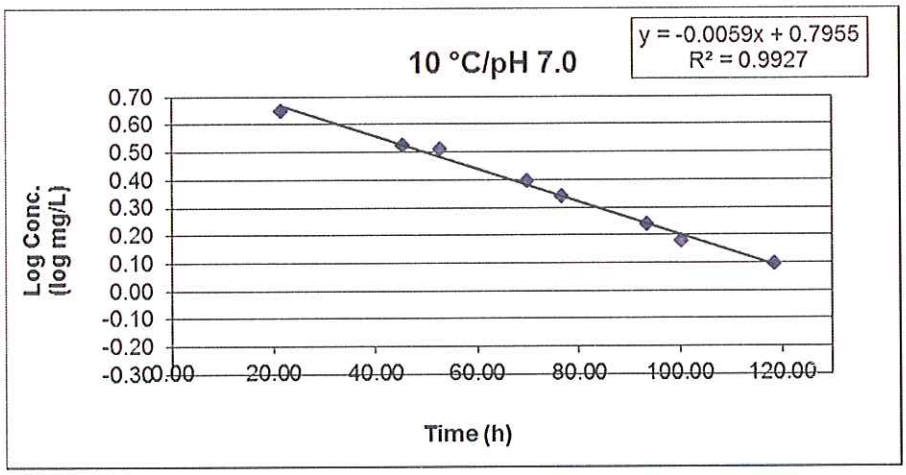
From the measured concentrations, the following percentage hydrolysis was calculated:

Table 9.2-g Hydrolysis pH 7, 10 °C

Sampling Time [h]	Recovery [%]	Decrease [%]	log C [log mg/L]
0.00	*100.0*	*0.0*	*0.6839*
21.58	91.9	-8.1	0.6474
45.58	69.4	-30.6	0.5250
52.75	66.7	-33.3	0.5079
69.92	51.6	-48.4	0.3962
76.75	45.1	-54.9	0.3385
93.50	35.6	-64.4	0.2355
100.17	31.3	-68.7	0.1790
118.50	25.9	-74.1	0.0969

The value, which is printed in *grey italics (0 h),* showed great deviation from the linear regression and was not used in the calculation of the hydrolysis function.

Graph of Hydrolysis (range 92 – 26 %):



Kinetic parameters were calculated from the regression linear using the equations given in chapter 8.3 (k_{obs} = (-2.303)*slope) and are presented in the following table:

Table 9.2-h Kinetic Parameters pH 7, 10 °C

Parameter	Value	Unit
Slope	-0.005939	log mg/L / h
k_{obs} (10 °C; pH 7.0)	0.013679	h^{-1}
$t_{1/2}$ (10 °C; pH 7.0)	50.7	h

9.2.1.3 pH 9.00
The values which were measured at 10 °C are presented in the following table:

Table 9.2-i Areas pH 9, 10 °C

Sampling Time [h]	Replicate 1		Replicate 2	
	Area 1 [pA*s]	Area 2 [pA*s]	Area 1 [pA*s]	Area 2 [pA*s]
0.00	226.29	232.31	231.28	229.64
20.75	216.29	216.06	193.52	219.28
44.75	183.27	173.70	159.78	160.70
52.08	173.30	177.39	173.82	175.82
69.25	145.55	141.17	157.95	159.23
76.08	134.94	141.20	132.13	142.94
92.83	108.18	104.61	*88.93*	*89.36*
117.92	76.22	75.37	73.67	75.42
164.50	*66.78*	*66.85*	47.97	48.29

The concentration of the test item at each sampling time was determined using the equation which is described in chapters 6.5.4.2 and 8.1, taking into account the recovery rate of the test item (89 %). Additionally, on days 5 (117.92 h) and 7 (164.5 h), the recovery rate of the respective QC sample (93 %) was taken into account, too.

Table 9.2-j Concentration (c_m) at pH 9 and 10 °C

Sampling Time	Factor	Replicate 1		Replicate 2		Conc. Mean
[h]		Conc. 1 [mg/L]	Conc. 2 [mg/L]	Conc. 1 [mg/L]	Conc. 2 [mg/L]	[mg/L]
0.00	0.1	4.80	4.93	4.91	4.88	4.88
20.75	0.1	4.58	4.58	4.08	4.65	4.47
44.75	0.1	3.86	3.65	3.34	3.36	3.55
52.08	0.1	3.64	3.73	3.65	3.69	3.68
69.25	0.1	3.03	2.93	3.30	3.33	3.15
76.08	0.1	2.79	2.93	2.73	2.97	2.86
92.83	0.1	2.20	2.13	*1.78*	*1.79*	2.17
117.92	0.1	1.61	1.59	1.55	1.59	1.59
164.50	0.1	*1.38*	*1.39*	0.94	0.95	0.95

The values, which are printed in *grey italics* (92.83 h, repl. 2 and 164.5 h, repl. 1), were not used in the calculation of the mean, as a mistake during the sample preparation (at 92.83 h) resp. a measuring error (164.5 h) were observed.

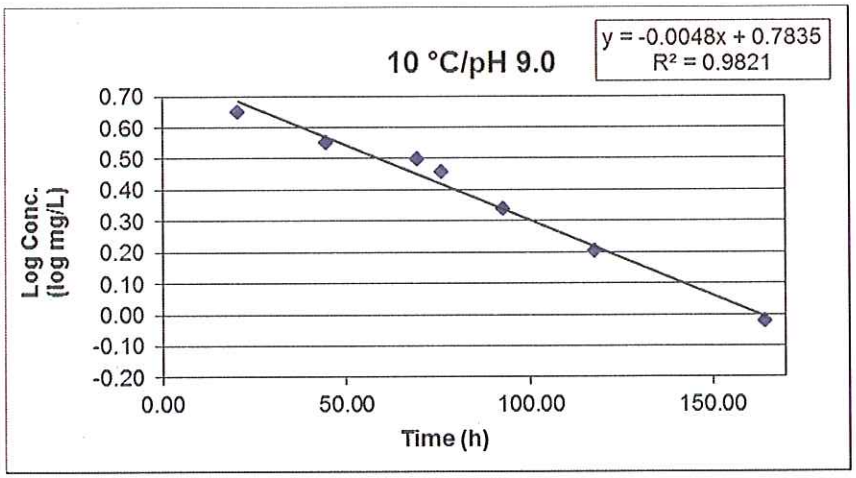
From the measured concentrations, the following percentage hydrolysis was calculated:

Table 9.2-k Hydrolysis pH 9, 10 °C

Sampling Time [h]	Recovery [%]	Decrease [%]	log C [log mg/L]
0.00	*100.0*	*0.0*	*0.6884*
20.75	91.6	-8.4	0.6503
44.75	72.7	-27.3	0.5502
52.08	*75.4*	*-24.6*	*0.5658*
69.25	64.5	-35.5	0.4983
76.08	58.6	-41.4	0.4564
92.83	44.5	-55.5	0.3365
117.92	32.6	-67.4	0.2014
164.50	19.5	-80.5	-0.0223

The values, which are printed in *grey italics* (0 h, 52.08 h), showed great deviation from the linear regression and were not used in the calculation of hydrolysis function.

Graph of Hydrolysis (range 92 – 20 %):



Kinetic parameters were calculated from the regression linear using the equations given in chapter 8.3 (k_{obs} = (-2.303)*slope) and are presented in the following table:

Table 9.2-l Kinetic Parameters pH 9, 10 °C

Parameter	Value	Unit
Slope	-0.004801	log mg/L / h
k_{obs} (10 °C; pH 9.0)	0.011056	h^{-1}
$t_{1/2}$ (10 °C; pH 9.0)	62.7	h

9.2.2 Test at 25 °C

9.2.2.1 pH 4.00

The first experiment was not used in the calculation of the kinetic parameters, as insufficient sampling points between 90 % and 10 % hydrolysis were measured. Therefore, the measured data are not included in this report but will be stored together with the other raw data of the study under GLP conditions.

The values which were measured at 25 °C in the second experiment are presented in the following table:

Table 9.2-m Areas pH 4, 25 °C

Sampling Time [h]	Replicate 1		Replicate 2	
	Area 1 [pA*s]	Area 2 [pA*s]	Area 1 [pA*s]	Area 2 [pA*s]
0.00	188.71	189.18	191.66	186.22
7.00	178.48	175.25	179.42	186.69
22.50	129.57	133.49	131.55	129.07
30.50	106.10	111.26	116.21	116.19
46.58	86.74	86.37	72.87	72.34
50.50	71.55	73.88	*104.48*	*101.07*
54.75	66.82	64.05	68.87	66.91
70.58	55.77	56.88	63.57	66.54
78.50	47.95	47.48	60.94	60.47

The concentration of the test item at each sampling time was determined using the equation which is described in chapters 6.5.4.2 and 8.1 taking into account the recovery rate of the test item (93 %). Additionally, the recovery rates of the respective QC samples were taken into account.

Table 9.2-n Concentration (c_m) at pH 4 and 25 °C

Sampling Time	Factor	Recovery Rate QC Sample	Replicate 1		Replicate 2		Conc. Mean
[h]		%	Conc. 1 [mg/L]	Conc. 2 [mg/L]	Conc. 1 [mg/L]	Conc. 2 [mg/L]	[mg/L]
0.00	0.1	93 %	4.07	4.08	4.14	4.02	4.08
7.00	0.1	93 %	3.84	3.77	3.86	4.03	3.88
22.50	0.1	91 %	2.81	2.90	2.86	2.80	2.84
30.50	0.1	91 %	2.27	2.39	2.50	2.50	2.42
46.58	0.1	90 %	1.85	1.84	1.52	1.51	1.68
50.50	0.1	90 %	1.49	1.55	*2.26**	*2.18**	1.52
54.75	0.1	90 %	1.38	1.31	1.43	1.38	1.35
70.58	0.1	94 %	1.07	1.10	1.25	1.31	1.18
78.50	0.1	94 %	0.90	0.89	1.19	1.18	1.04

***Replicate 2 (50.5 h) was stated as outlier and was not used in the calculation of the mean**

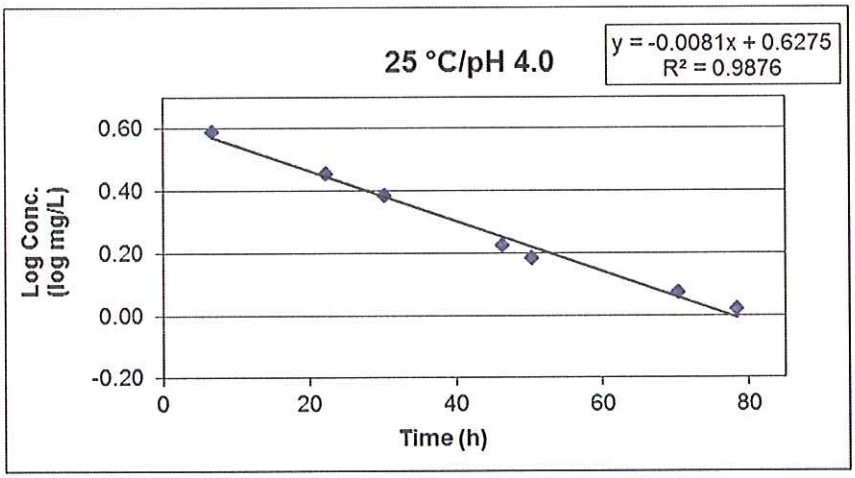
From the measured concentrations, the following percentage hydrolysis was calculated:

Table 9.2-o Hydrolysis pH 4, 25 °C

Sampling Time [h]	Recovery [%]	Decrease [%]	log C [log mg/L]
0.00	*100.0*	*0.0*	*0.6107*
7.00	95.1	-4.9	0.5888
22.50	69.6	-30.4	0.4533
30.50	59.3	-40.7	0.3838
46.58	41.2	-58.8	0.2253
50.50	37.3	-62.7	0.1818
54.75	*33.1*	*-66.9*	*0.1303*
70.58	28.9	-71.1	0.0719
78.50	25.5	-74.5	0.0170

The values, which are printed in *grey italics* (0 h, 54.75 h), showed great deviation from the linear regression and were not used in the calculation of the hydrolysis function.

Graph of Hydrolysis (range 95 – 26 %):



Kinetic parameters were calculated from the regression linear using the equations given in chapter 8.3 (k_{obs} = (-2.303)*slope) and are presented in the following table:

Table 9.2-p Kinetic Parameters pH 4, 25 °C

Parameter	Value	Unit
Slope	-0.008070	log mg/L / h
k_{obs} (25 °C; pH 4.0)	0.018586	h^{-1}
$t_{1/2}$ (25 °C; pH 4.0)	37.3	h

9.2.2.2 pH 7.00
The values which were measured at 25 °C are presented in the following table:

Table 9.2-q Areas pH 7, 25 °C

Sampling Time [h]	Replicate 1		Replicate 2	
	Area 1 [pA*s]	Area 2 [pA*s]	Area 1 [pA*s]	Area 2 [pA*s]
0.00	221.13	227.08	224.99	233.79
17.50	175.12	178.97	174.12	-*
24.67	165.32	172.68	162.82	169.00
43.17	112.09	*36.92*	124.72	121.37
89.50	54.39	55.04	57.47	59.17
95.83	46.51	45.97	47.62	47.32
112.50	43.08	43.44	34.05	35.10
119.83	38.46	34.88	34.76	33.26

***Due to an instrumental failure, the chromatogram was not available**

The concentration of the test item at each sampling time was determined using the equation which is described in chapters 6.5.4.2 and 8.1 taking into account the recovery rate of the test item (92 %). Additionally, the recovery rates of the respective QC samples were taken into account.

Table 9.2-r Concentration (c_m) at pH 7 and 25 °C

Sampling Time	Factor	Recovery Rate QC Sample	Replicate 1		Replicate 2		Conc. Mean
[h]		%	Conc. 1 [mg/L]	Conc. 2 [mg/L]	Conc. 1 [mg/L]	Conc. 2 [mg/L]	[mg/L]
0.00	0.1	-	4.54	4.66	4.62	4.81	4.66
17.50	0.1	91 %	3.90	3.99	3.88	< LOD	3.92
24.67	0.1	91 %	3.68	3.85	3.62	3.76	3.73
43.17	0.1	93 %	2.39	*0.66*	2.68	2.60	2.56
89.50	0.1	91 %	1.09	1.10	1.16	1.20	1.14
95.83	0.1	91 %	0.90	0.89	0.93	0.92	0.96
112.50	0.1	93 %	0.81	0.81	0.60	0.62	0.71
119.83	0.1	93 %	0.70	0.62	0.62	0.58	0.63

The value, which is printed in *grey italics* (43.17 h, repl. 1-2), showed great deviation from the other measurements and was not used in the calculation of the mean.

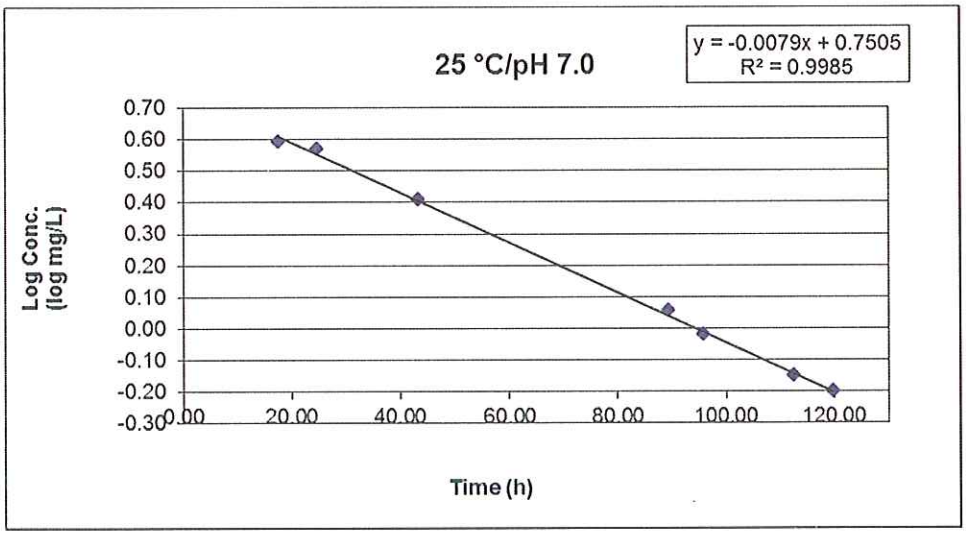
From the measured concentrations, the following percentage hydrolysis was calculated:

Table 9.2-s Hydrolysis pH 7, 25 °C

Sampling Time [h]	Recovery [%]	Decrease [%]	log C [log mg/L]
0.00	*100.0*	*0.0*	*0.6684*
17.50	84.1	-15.9	0.5933
24.67	80.0	-20.0	0.5717
43.17	54.9	-45.1	0.4082
89.50	24.5	-75.5	0.0569
95.83	20.6	-79.4	-0.0177
112.50	15.2	-84.8	-0.1487
119.83	13.5	-86.5	-0.2007

The value, which is printed in *grey italics* (0 h), showed great deviation from the linear regression and was not used in the calculation of the hydrolysis function.

Graph of Hydrolysis (range 84 – 14 %):



Kinetic parameters were calculated from the regression linear using the equations given in chapter 8.3 (k_{obs} = (-2.303)*slope) and are presented in the following table:

Table 9.2-t Kinetic Parameters pH 7, 25 °C

Parameter	Value	Unit
Slope	-0.007933	log mg/L / h
k_{obs} (10 °C; pH 7.0)	0.018271	h^{-1}
$t_{1/2}$ (10 °C; pH 7.0)	37.9	h

9.2.2.3 pH 9.00
The values which were measured at 25 °C are presented in the following table:

Table 9.2-u Areas pH 9, 25 °C

Sampling Time [h]	Replicate 1		Replicate 2	
	Area 1 [pA*s]	Area 2 [pA*s]	Area 1 [pA*s]	Area 2 [pA*s]
0.00	213.57	221.18	214.71	210.74
17.50	167.08	172.83	170.23	172.06
24.67	173.74	174.15	178.14	175.98
43.17	133.12	127.57	123.18	123.56
89.50	73.62	78.63	54.52	56.47
95.83	59.58	61.09	48.63	50.62
112.50	57.88	61.47	51.23	55.70
119.83	46.79	46.08	-	-

The concentration of the test item at each sampling time was determined using the equation which is described in chapters 6.5.4.2 and 8.1 taking into account the recovery rate of the test item (89 %). Additionally, the recovery rates of the respective QC samples were taken into account.

Table 9.2-v Concentration (c_m) at pH 9 and 25 °C

Sampling Time	Factor	Recovery Rate QC Sample	Replicate 1		Replicate 2		Conc. Mean c_m
[h]		%	Conc. 1 [mg/L]	Conc. 2 [mg/L]	Conc. 1 [mg/L]	Conc. 2 [mg/L]	[mg/L]
0.00	0.1	-	4.52	4.69	4.55	4.46	4.56
17.50	0.1	91 %	3.84	3.98	3.92	3.96	3.93
24.67	0.1	91 %	4.00	4.01	4.11	4.06	4.05
43.17	0.1	93 %	2.97	2.84	2.73	2.74	2.82
89.50	0.1	91 %	1.59	1.71	1.13	1.18	1.40
95.83	0.1	91 %	1.25	1.29	0.99	1.03	1.10
112.50	0.1	93 %	1.19	1.27	1.03	1.13	1.16
119.83	0.1	93 %	0.92	0.91	-*	-*	0.92

***After 119.83 h, one replicate was used for extraction only, as the second replicate was broken**

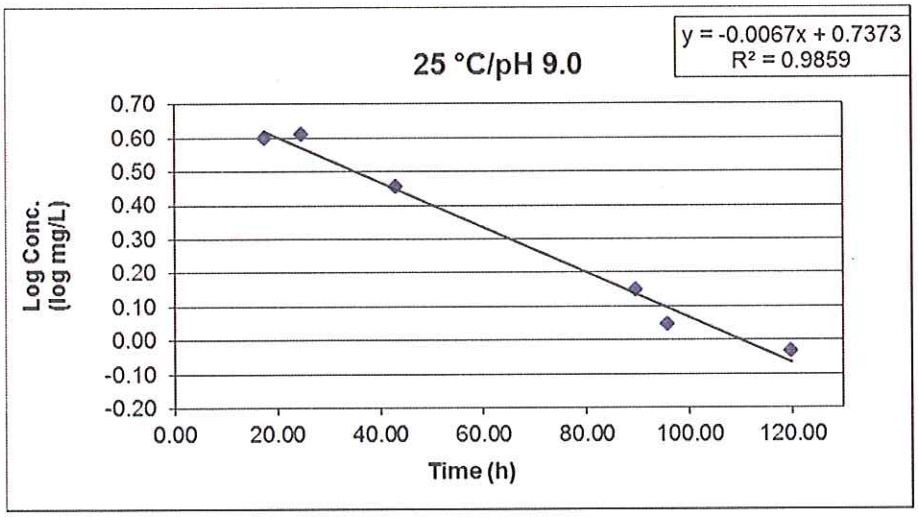
From the measured concentrations, the following percentage hydrolysis was calculated:

Table 9.2-w Hydrolysis pH 9, 25 °C

Sampling Time [h]	Recovery [%]	Decrease [%]	log C [log mg/L]
0.00	*100.0*	*0.0*	*0.6590*
17.50	86.2	-13.8	0.5944
24.67	88.8	-11.2	0.6075
43.17	61.8	-38.2	0.4502
89.50	30.7	-69.3	0.1461
95.83	24.1	-75.9	0.0414
112.50	*25.4*	*-74.6*	*0.0645*
119.83	20.2	-79.8	-0.0362

The values, which are printed in *grey italics* (0 h, 112.5 h), showed great deviation from the linear regression and were not used in the calculation of the hydrolysis function.

Graph of Hydrolysis (range 86 – 20 %):



Kinetic parameters were calculated from the regression linear using the equations given in chapter 8.3 (k_{obs} = (-2.303)*slope) and are presented in the following table:

Table 9.2-x Kinetic Parameters pH 9, 25 °C

Parameter	Value	Unit
Slope	-0.006711	log mg/L / h
k_{obs} (25 °C; pH 9.0)	0.015455	h^{-1}
$t_{1/2}$ (25 °C; pH 9.0)	44.8	h

9.2.1 Test at 50 °C

9.2.1.1 pH 4.00

The values which were measured at 50 °C are presented in the following table:

Table 9.2-y Areas pH 4, 50 °C

Sampling Time [h]	Replicate 1		Replicate 2	
	Area 1 [pA*s]	Area 2 [pA*s]	Area 1 [pA*s]	Area 2 [pA*s]
0.00	216.18	207.03	226.60	224.79
2.75	211.88	208.19	208.37	217.00
5.00	198.79	201.23	207.89	212.38
7.75	178.95	178.43	180.03	174.71
23.00	90.43	88.19	95.75	99.60
26.00	123.52	121.23	107.21	117.02
29.00	78.13	87.89	78.07	76.94
31.75	89.92	83.11	81.48	77.03
34.50	70.08	69.69	90.20	85.35
47.25	67.44	71.62	-*	-*

***After 47.25 h, one replicate was used for extraction only, as the second replicate was broken**

The concentration of the test item at each sampling time was determined using the equation which is described in chapters 6.5.4.4 and 8.1 taking into account the recovery rate of the test item (93 %).

Table 9.2-z Concentration (c_m) at pH 4 and 50 °C

Sampling Time	Factor	Replicate 1		Replicate 2		Conc. Mean
[h]		Conc. 1 [mg/L]	Conc. 2 [mg/L]	Conc. 1 [mg/L]	Conc. 2 [mg/L]	[mg/L]
0.00	0.1	5.34	5.13	5.58	5.54	5.40
2.75	0.1	5.24	5.16	5.16	5.36	5.23
5.00	0.1	4.94	5.00	5.15	5.26	5.09
7.75	0.1	4.48	4.47	4.50	4.38	4.46
23.00	0.1	2.29	2.23	2.43	2.53	2.37
26.00	0.1	3.13	3.08	2.72	2.97	2.98
29.00	0.1	1.97	2.23	1.97	1.94	2.03
31.75	0.1	2.28	2.10	2.06	1.94	2.10
34.50	0.1	1.76	1.75	2.29	2.16	1.99
47.25	0.1	1.69	1.80	-	-	1.75

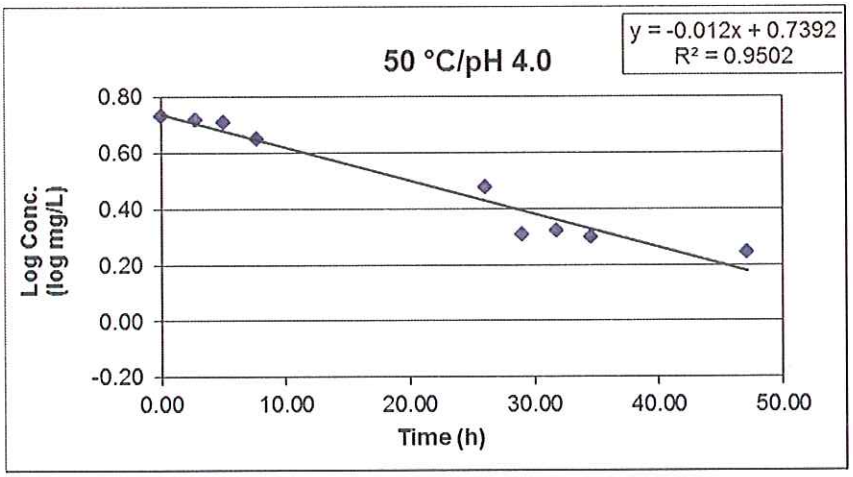
From the measured concentrations, the following percentage hydrolysis was calculated:

Table 9.2-aa Hydrolysis pH 4, 50 °C

Sampling Time [h]	Recovery [%]	Decrease [%]	log C [log mg/L]
0.00	100.0	0.0	0.7324
2.75	96.9	-3.1	0.7185
5.00	94.3	-5.7	0.7067
7.75	82.6	-17.4	0.6493
23.00	*43.9*	*-56.1*	*0.3747*
26.00	55.2	-44.8	0.4742
29.00	37.6	-62.4	0.3075
31.75	38.9	-61.1	0.3222
34.50	36.9	-63.1	0.2989
47.25	32.4	-67.6	0.2430

The values, which are printed in *grey italics* (23 h), showed great deviation from the linear regression and was not used in the calculation of the hydrolysis function.

Graph of Hydrolysis (range 100 – 32 %):



Kinetic parameters were calculated from the regression linear using the equations given in chapter 8.3 (k_{obs} = (-2.303)*slope) and are presented in the following table:

Table 9.2-bb Kinetic Parameters pH 4, 50 °C

Parameter	Value	Unit
Slope	-0.011960	log mg/L / h
k_{obs} (50 °C; pH 4.0)	0.027544	h^{-1}
$t_{1/2}$ (50 °C; pH 4.0)	25.2	h

9.2.1.2 pH 7.00
The values which were measured at 50 °C are presented in the following table:

Table 9.2-cc Areas pH 7, 50 °C

Sampling Time [h]	Replicate 1		Replicate 2	
	Area 1 [pA*s]	Area 2 [pA*s]	Area 1 [pA*s]	Area 2 [pA*s]
0.00	183.10	174.32	196.02	187.01
8.08	176.76	161.88	172.16	179.36
10.33	158.34	151.42	164.16	161.70
22.92	127.85	125.23	109.77	124.40
26.08	114.38	125.67	129.39	135.81
31.08	115.49	115.26	105.91	104.99
47.00	75.09	79.47	77.39	70.98
54.08	*94.19*	*88.95*	67.19	71.51
70.83	48.44	43.25	*87.23*	*88.08*

The concentration of the test item at each sampling time was determined using the equation which is described in chapters 6.5.4.4 and 8.1 taking into account the recovery rate of the test item 92 %.

Table 9.2-dd Concentration (c_m) at pH 7 and 50 °C

Sampling Time	Factor	Replicate 1		Replicate 2		Conc. Mean
[h]		Conc. 1 [mg/L]	Conc. 2 [mg/L]	Conc. 1 [mg/L]	Conc. 2 [mg/L]	[mg/L]
0.00	0.1	4.63	4.42	4.93	4.72	4.68
8.08	0.1	4.48	4.12	4.36	4.54	4.38
10.33	0.1	4.03	3.86	4.17	4.11	4.04
22.92	0.1	3.28	3.21	2.82	3.19	3.13
26.08	0.1	2.94	3.22	3.32	3.48	3.24
31.08	0.1	2.96	2.96	2.72	2.70	2.84
47.00	0.1	1.91	2.03	1.97	1.80	1.93
54.08	0.1	*2.42*	*2.28*	1.70	1.82	1.76
70.83	0.1	1.19	1.05	*2.23*	*2.26*	1.12

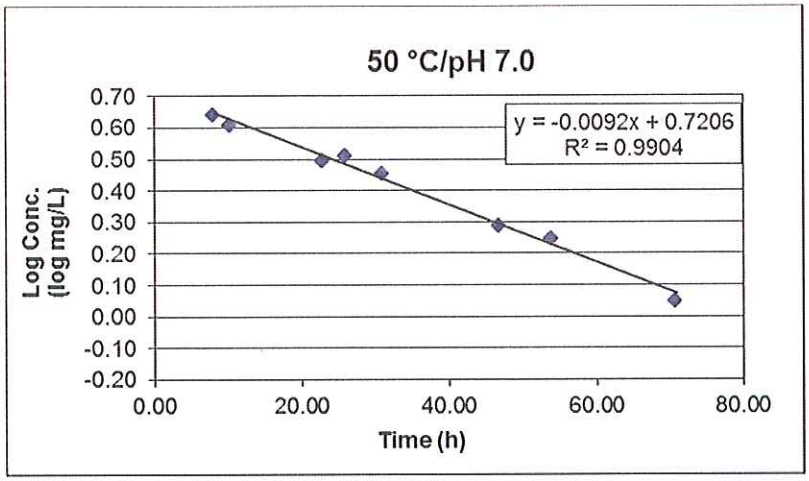
The value, which is printed in *grey italics* (*54* h, repl. 1 and 70.83 h, repl. 2), showed great deviation from the linear regression and was not used in the calculation of the mean.
From the measured concentrations, the following percentage hydrolysis was calculated:

Table 9.2-ee Hydrolysis pH 7, 50 °C

Sampling Time [h]	Recovery [%]	Decrease [%]	log C [log mg/L]
0.00	*100.0*	*0.0*	*0.6702*
8.08	93.6	-6.4	0.6415
10.33	86.3	-13.7	0.6064
22.92	66.9	-33.1	0.4955
26.08	69.2	-30.8	0.5105
31.08	60.7	-39.3	0.4533
47.00	41.2	-58.8	0.2856
54.08	37.6	-62.4	0.2455
70.83	23.9	-76.1	0.0492

The value, which is printed in *grey italics* (0 h), showed great deviation from the linear regression and was not used in the calculation of the hydrolysis function.

Graph of Hydrolysis (range 94 – 24 %):



Kinetic parameters were calculated from the regression linear using the equations given in chapter 8.3 (k_{obs} = (-2.303)*slope) and are presented in the following table:

Table 9.2-ff Kinetic Parameters pH 7, 50 °C

Parameter	Value	Unit
Slope	-0.009159	log mg/L / h
k_{obs} (10 °C; pH 7.0)	0.021094	h^{-1}
$t_{1/2}$ (10 °C; pH 7.0)	32.9	h

9.2.1.3 pH 9.00
The values which were measured at 50 °C are presented in the following table:

Table 9.2-gg Areas pH 9, 50 °C

Sampling Time [h]	Replicate 1		Replicate 2	
	Area 1 [pA*s]	Area 2 [pA*s]	Area 1 [pA*s]	Area 2 [pA*s]
0.00	206.31	205.77	200.38	204.17
7.27	172.00	175.01	160.54	168.63
9.52	148.75	151.24	144.93	141.12
22.10	77.20	73.61	67.25	65.93
25.27	47.74	47.31	52.30	49.72
27.77	45.13	40.86	47.69	47.70
29.27	42.05	41.81	38.66	36.01
46.18	7.04	7.63	9.88	10.09

The concentration of the test item at each sampling time was determined using the equation which is described in chapters 6.5.4.4 and 8.1 taking into account the recovery rate of the test item (89 %).

Table 9.2-hh Concentration (c_m) at pH 9 and 50 °C

Sampling Time	Factor	Replicate 1		Replicate 2		Conc. Mean c_m
[h]		Conc. 1 [mg/L]	Conc. 2 [mg/L]	Conc. 1 [mg/L]	Conc. 2 [mg/L]	[mg/L]
0.00	0.1	5.35	5.33	5.20	5.30	5.30
7.27	0.1	4.51	4.58	4.22	4.42	4.43
9.52	0.1	3.92	3.99	3.83	3.73	3.87
22.10	0.1	2.04	1.94	1.76	1.72	1.87
25.27	0.1	1.21	1.20	1.34	1.27	1.26
27.77	0.1	1.14	1.02	1.21	1.21	1.15
29.27	0.1	1.05	1.04	0.95	0.88	0.98
46.18	0.1	0.02	0.04	0.11	0.11	0.07

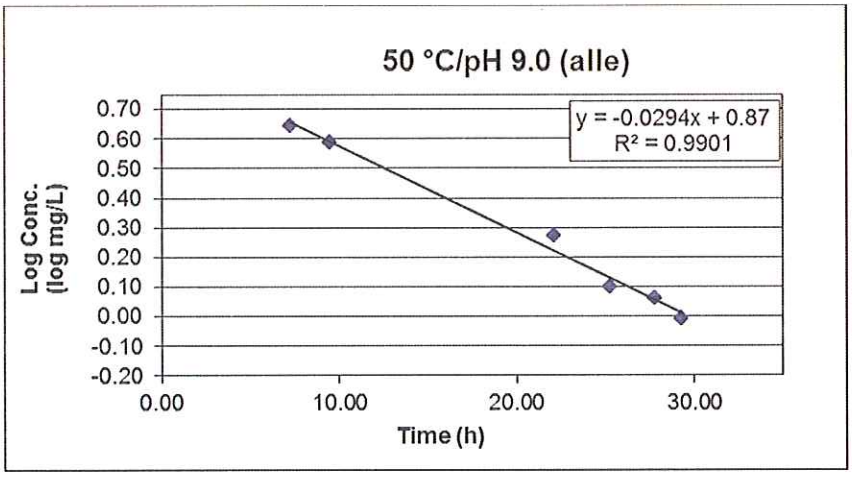
From the measured concentrations, the following percentage hydrolysis was calculated:

Table 9.2-ii Hydrolysis pH 9, 50 °C

Sampling Time [h]	Recovery [%]	Decrease [%]	log C [log mg/L]
0.00	*100.0*	*0.0*	*0.7243*
7.27	83.6	-16.4	0.6464
9.52	73.0	-27.0	0.5877
22.10	35.3	-64.7	0.2718
25.27	23.8	-76.2	0.1004
27.77	21.7	-78.3	0.0607
29.27	18.5	-81.5	-0.0088
46.18	*1.3*	*-98.7*	*-1.1549*

The values, which are printed in *grey italics* (0 h, 46.18 h), showed great deviation from the linear regression and were not used in the calculation of the hydrolysis function.

Graph of Hydrolysis (range 84 – 19 %):



Kinetic parameters were calculated from the regression linear using the equations given in chapter 8.3 (k_{obs} = (-2.303)*slope) and are presented in the following table:

Table 9.2-jj Kinetic Parameters pH 9, 50 °C

Parameter	Value	Unit
Slope	-0.029389	log mg/L / h
k_{obs} (25 °C; pH 9.0)	0.067684	h^{-1}
$t_{1/2}$ (25 °C; pH 9.0)	10.2	h

An additional signal was observed in GC-chromatogram (approx. 2.6 min.) at pH 9 only.

9.2.2 Results - Overview

The following hydrolysis constants and half-lives were determined at the three pH values and the three temperatures:

Table 9.2-kk Results Tier 2

Temperature [°C]	pH	K_{obs} [h^{-1}] (pH)	Half-life [h]	K_{obs} [h^{-1}] (total)	Half-life [h] (total)
50	4.00	0.027544	25.2	0.116322	5.96
	7.00	0.021094	32.9		
	9.00	0.067684	10.2		
25	4.00	0.018586	37.3	0.052312	13.25
	7.00	0.018271	37.9		
	9.00	0.015455	44.8		
10	4.00	0.014682	47.2	0.039416	17.59
	7.00	0.013679	50.7		
	9.00	0.011056	62.7		

Each K_{obs} (total) was calculated as sum of the experimentally determined constants k_{obs} for each temperature. Half-life was calculated from ln (2)/k.

9.2.3 Temperature Dependency following Arrhenius

Using the experimentally determined k_{obs} for each pH and temperature, temperature dependency was calculated, using the relation ln k vs. 1/T. The values are given in the following table:

Table 9.2-II Temperature dependency of ln k

Temperature [°C]	Temperature [1/K]	pH	k_{obs} (pH)	ln k_{obs}
50	0.003096	4.00	0.027544	-3.592
		7.00	0.021094	-3.859
		9.00	0.067684	-2.693
25	0.003356	4.00	0.018586	-3.985
		7.00	0.018271	-4.002
		9.00	0.015455	-4.170
10	0.003534	4.00	0.014682	-4.221
		7.00	0.013679	-4.292
		9.00	0.011056	-4.505

The values were plotted and slope and intercept were determined for all pH values.

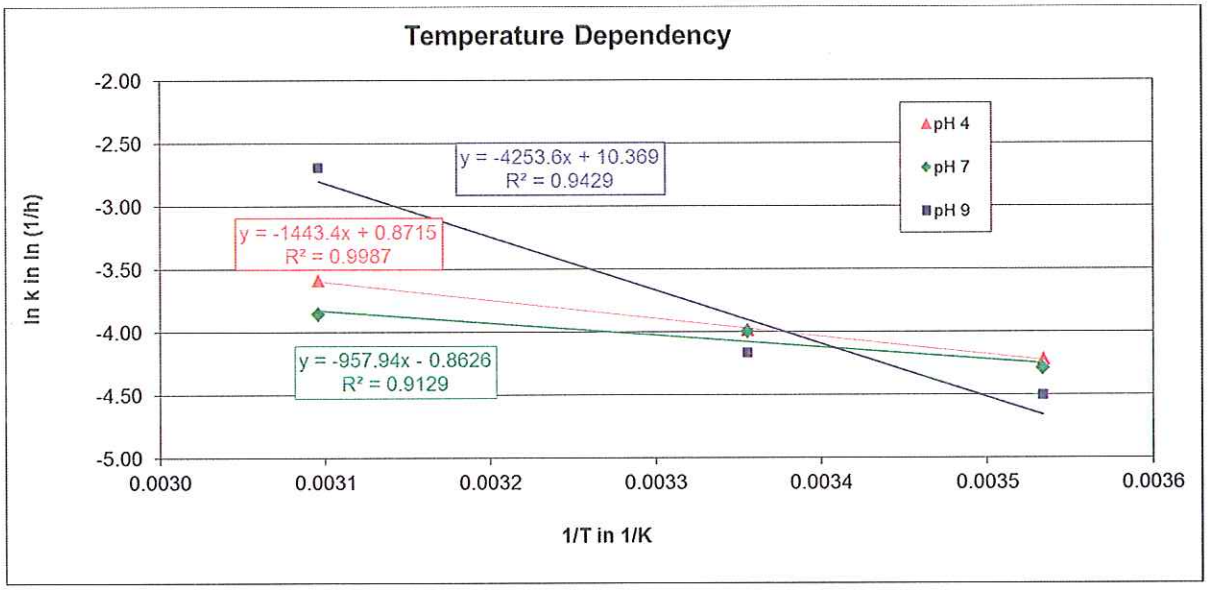


Table 9.2-mm Regression Parameters

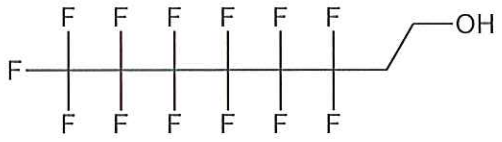
Parameter	pH 4	pH 7	pH 9
Slope	-1443	-958	-4254
Intercept	0.87	-0.86	10.37
Coefficient of Determination r^2	0.9987	0.9129	0.9429

With these parameters, k_{obs} (total) and half-life at 20 °C were calculated, using the ARRHENIUS equation with:

$$K_{obs} = e^{0.87} * e^{-1443/293} + e^{-0.87} * e^{-954/293} + e^{10.37} * e^{-4254/293} = 0.04917 \text{ correlating to } t_{1/2} = 14.1 \text{ h}$$

9.3 Tier 3

One additional signal at 2.6 minutes was observed after the hydrolysis of the test item at pH 9. The peak was identified via GC/MS analysis in the laboratory Dr. Appelt in Mannheim under non-GLP conditions as the expected product of alkaline hydrolysis 3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctyl alcohol:



FC(F)(C(F)(F)CCO)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F

10 Discussion

Hydrolysis behaviour of the test item 3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctyl acrylate was examined at three different pH values and three different temperatures. At 10 °C, 25 °C and 50 °C, hydrolysis was completed within 1 – 5 days. Therefore, the test item can be considered as hydrolytically instable at all pH values 4, 7 and 9.

Ester decomposition is usually catalysed both by acids and bases. The test item 3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctyl acrylate seems to be affected much more quickly by acids at 10 °C and 25 °C, but at 50 °C the hydrolysis was quicker at pH 9. An additional signal was observed in the GC-chromatogram (at approx. 2.6 min.) at pH 9 only and was identified via GC/MS analysis in the laboratory Dr. Appelt in Mannheim under non-GLP conditions as 3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctyl alcohol.

Hydrolysis rates are higher with increasing temperature.

All validity criteria for analytical method and determination of hydrolysis were met; repeatability of the values was very good, and coefficients of determination all lay above 0.95.

Temperature dependencies of the hydrolysis constants fit very well the Arrhenius equation indicating that the reaction is pseudo-first order indeed.

11 Deviations

11.1 Deviations from the Study Plan

The following deviations from the study plan were documented:

- Argon was used without pre-treatment with dithionite solution. This was considered as uncritical as Argon p.A. was used and based on experience no difference in the results was observed.
- Identification of hydrolysis products was not performed for pHs 4 and 7, as no additional product was observed during hydrolysis at pH 4 and 7.

The deviations were signed and assessed by the study director on 04. Mar. 2013.

11.2 Deviations from the Guideline

No deviations from the guideline were stated.

12 Recording

One original of study plan and final report, respectively, all raw data of the study and all documents mentioned or referred to in study plan or final report will be kept in the GLP Document Archive of the test facility for fifteen years. After that, the sponsor's instructions will be applied (destruction of documentation). A retain sample of the test item will be kept in the GLP Substance Archive for fifteen years; then, the retain sample will be discarded.

Number of originals which will be sent to the sponsor: 1

13 ANNEX 1: COPY OF GLP-CERTIFICATE

RheinlandPfalz
LANDESAMT FÜR UMWELT, WASSERWIRTSCHAFT UND GEWERBEAUFSICHT

GUTE LABORPRAXIS – GOOD LABORATORY PRACTICE

GLP-BESCHEINIGUNG
STATEMENT OF GLP COMPLIANCE

gemäß/according to § 19b Abs. 1 Chemikaliengesetz

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 2004/9/EG wurde durchgeführt in:

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 2004/9/EC at:

Prüfeinrichtung / Test facility

LAUS GmbH
Auf der Schafweide 20
67489 Kirrweiler

Prüfung nach Kategorien / Areas of Expertise
(gemäß / according ChemVwV-GLP Nr. 5.3/OECD guidance)
1, 3, 4, 5, 6 , 8, 9 (toxikologische in Vitro Prüfungen an Säugerzellen und Bakterien)

Datum der Inspektion / Date of Inspection
(Tag.Monat.Jahr / day.month.year)
29. und 30. November 2010

Die genannte Prüfeinrichtung befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

The above mentioned test facility is included in the national GLP Compliance Programme and is inspected on a regular basis.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.

Based on the inspection report it can be confirmed, that the test facility is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Eine erneute behördliche Überprüfung der Einhaltung der GLP-Grundsätze durch die Prüfeinrichtung ist so rechtzeitig zu beantragen, dass die Folgeinspektion spätestens vier Jahre nach dem Beginn der o.g. Inspektion stattfinden kann. Ohne diesen Antrag wird die Prüfeinrichtung nach Ablauf der Frist aus dem deutschen GLP-Überwachungsprogramm genommen und diese GLP-Bescheinigung verliert ihre Gültigkeit.

Verification of the compliance of the test facility with the Principles of the GLP has to be applied for in time to allow for a follow-up inspection to take place within four years after commencing the above mentioned inspection. Elapsing this term, the test facility will be taken out of the German GLP-Monitoring Programme and this GLP Certificate becomes invalid.

Unterschrift, Datum / Signature, Date

11.04.11

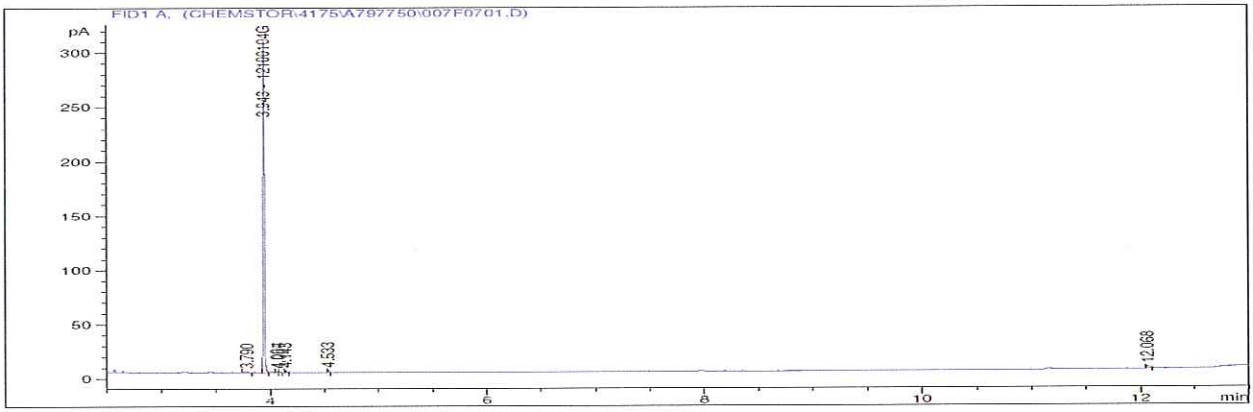
Dr.-Ing. Stefan Hill - Präsident -
(Name und Funktion der verantwortlichen Person / name and function of responsible person)

Landesamt für Umwelt, Wasserwirtschaft und Gewerbeaufsicht
Kaiser-Friedrich-Straße 7, 55116 Mainz
(Name und Adresse der GLP-Überwachungsbehörde / Name and adress of the GLP Monitoring Authority)

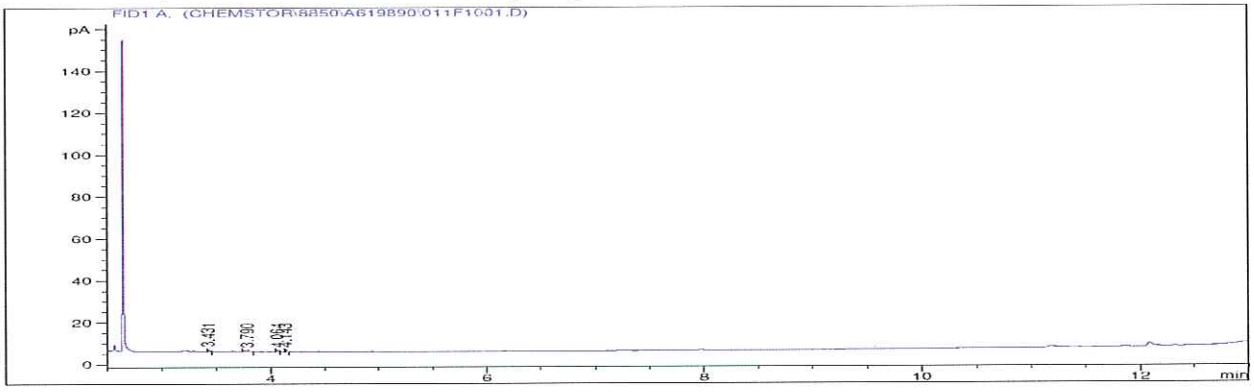
MESSEN BEWERTEN BERATEN

14 ANNEX 2: CHROMATOGRAMS

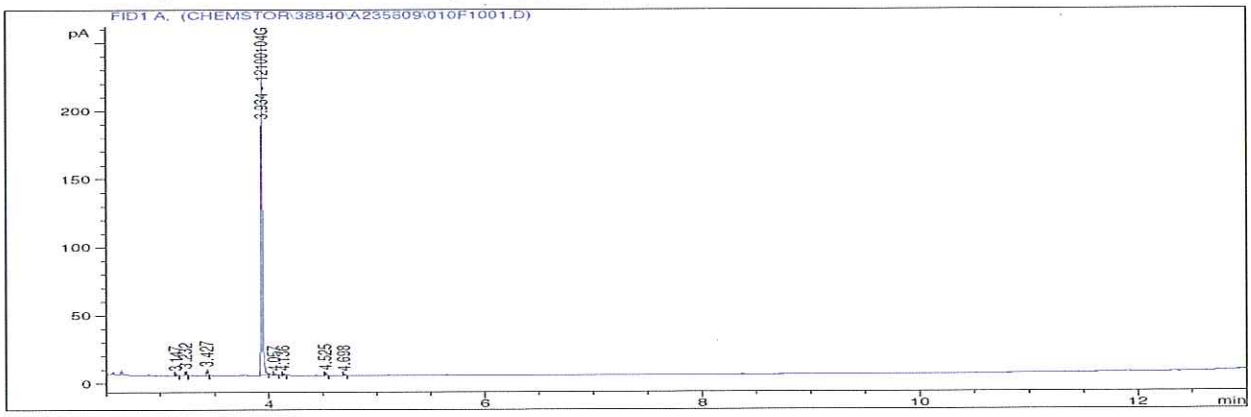
14.1 Tier 1, Test Item Solution, day 0, pH 9



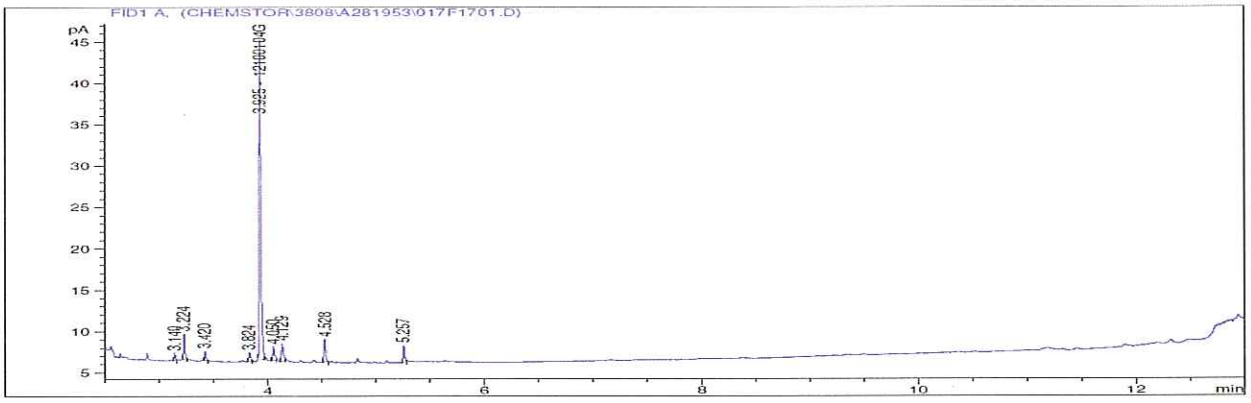
14.2 Tier 1, Test Item Solution, day 5, pH 9, 50 °C



14.3 Tier 2, Test Item Solution, 0 h, pH 7, 25°C



14.4 Tier 2, Test Item Solution, 120 h, pH 7, 25°C



15 ANNEX 3: GC/MS ANALYSIS (TIER 3)

GC/MS Method

Analysis Instruments
Injector/sampler: Gerstel KAS4/MPS2L
GC: Agilent 6890
MS: Agilent 5975

GC Oven Program
50 °C for 1 min
then 10 °C/min to 100 °C for 1 min
then 8 °C/min to 172 °C for 0 min
then 40 °C/min to 240 °C for 2 min
Run time 19.7 min

GC to MS Transfer Line
Temperature 240 °C

Capillary Column
Restek Rtx-624
30 m x 250 µm x 1.4 µm

GC-Injector
Carrier gas	Helium
Mode	total injection with solvent vent
Injektor temp	50 °C to 240 °C
Pressure mode	Constant flow
Flow	1.8 mL/min
Split ratio	50:1
Inj. volume	3 µL

MS Parameters
Ionization	EI 70 eV
Mode	scan m/z 35 – 350

GC/MS Data Evaluation
Signal used for peak integration Total Ion Current m/z 35 - 350
Mass spectra database Nist08