Final Report

Original 2 of 2

Determination of the aerobic ready biodegradability of
PRO-SHAR PAINTBALLS
in the CO_2 Evolution Test
following OECD 301B resp. EU C.4-C

Study No.: 17040403G605

Sponsor:
PRO-SHAR EUROPE BV
SATELLIETBAAN 15
NL-2181 MG HILLEGOM
THE NETHERLANDS

Monitor:
David van der Plas

Test Facility:
LAUS GmbH
Auf der Schafweide 20
D-67489 Kirrweiler, Germany

Study Director:
Manfred Muckle

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)
- Chemicals Act of the Federal Republic of Germany (ChemG) §19a and §19b and annexes 1 and 2 from 28. Aug. 2013, published in Federal Law Gazette, Germany (BGBl) No. 55/2013 as of 06. Sep. 2013, and further revisions.

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.

Manfred Muckle
Study Director

08 FEB 2018
Date

Information on Study Organisation:

Deputy Study Director	Elke Klein
Study Plan dated	18. Aug. 2017
Experimental Starting Date	04. Oct. 2017
Experimental Completion Date	08. Nov. 2017

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	16. Aug. 2017	16. Aug. 2017	170816-12
Performance of study	09. Oct. 2017	09. Oct. 2017	171009-08
Draft report	18. Dec. 2017	18. Dec. 2017	171218-04
Final report	06. Feb. 2018	06. Feb. 2018	180206-08

Revina-Rosa Resch
Quality Assurance

08 FEB 2018
Date

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3 SUMMARY

Title of Study: Determination of the aerobic ready biodegradability of PRO-SHAR PAINTBALLS in the CO_2 Evolution Test following OECD 301B resp. EU C.4-C

Findings and Results:

The test item PRO-SHAR PAINTBALLS was tested using a concentration of nominally 20 mg organic carbon/L (corresponding to 41.3 mg PRO-SHAR PAINTBALLS/L) in test medium following OECD 301B and EU-Method C.4-C.

Aniline was chosen as positive control.

Activated sludge was used as inoculum (concentration in the test 25.0 mg dry matter/L). The test was left running for 28 days.

All validity criteria were met. Degradation of the positive control was 69 % after 10 days.

The following data were determined for the test item PRO-SHAR PAINTBALLS:

10-day-window:	day 5 – 15
degradation at the end of 10-day-window	78 %
degradation at the end of the test	84 %
pass level following guideline:	60 % at the end of 10-day-window for pure substances respectively 60 % at the end of the test for mixtures

Therefore, when applying the 10-day-window, PRO-SHAR PAINTBALLS is **readily biodegradable** following OECD 301B and EU C.4-C respectively.
Because the test item is a mixture the 10-day-window has not to be taken into account.
Regardless of the 10-day-window, PRO-SHAR PAINTBALLS is **readily biodegradable** following OECD 301B and EU C.4-C respectively.

4 PURPOSE AND PRINCIPLE OF THE STUDY

This study was performed in order to evaluate aerobic elimination and degradation potential of PRO-SHAR PAINTBALLS in a test for ready biodegradability, using a test item concentration of nominally 20 mg organic carbon/L (corresponding to 41.3 mg PRO-SHAR PAINTBALLS/L).

The test item in a mineral medium was inoculated and incubated under aerobic conditions in the dark. The amount of DOC in the test solution due to the inoculum was kept as low as possible compared with the amount of organic carbon due to the test item. Allowance was made for the endogenous activity of the inoculum by running parallel blanks with inoculum but without test item. A positive control was run in parallel to check the operation of the procedures. Degradation was followed by determining the carbon dioxide produced. Measurements were taken at sufficiently frequent intervals to allow the identification of the beginning and end of biodegradation.

The test lasted for 28 days.

5 LITERATURE

The study was conducted in accordance with the following guidelines:

- OECD Guidelines for the Testing of Chemicals, Part 301 B, adopted 17. Jul. 1992 "CO_2-Evolution-Test (Modified STURM Test)"
- Council Regulation (EC) No. 440/2008, Method C.4-C, adopted 30. May 2008 "CO_2-Evolution-Test"

Corresponding SOP of LAUS GmbH:

- SOP 118 006 05 edition 10, valid from 25. Apr. 2016 „Abbaubarkeitstest nach OECD 301B / EU C.4-C"

6 MATERIALS AND METHODS

6.1 Test Item

Designation in Test Facility: 17040403G
Date of Receipt: 04. Apr. 2017
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	PRO-SHAR PAINTBALLS
Batch no.	4936
Appearance	Soft gelatin capsules containing colored liquid
Composition	Gelatin, Glycerol, Organic Dyes, Titanium Dioxide, PEG 400, PEG 4000, Water
Purity	not applicable, mixture
Homogeneity	homogeneous
Vapour pressure	unknown
Stability	H_2O: unknown; EtOH: unknown; acetone: unknown; CH_3CN: unknown; DMSO: unknown
Solubility	H_2O: not stated; EtOH: not stated; acetone: not stated; CH_3CN: not stated; DMSO: not stated
Production date	01. Feb. 2017
Expiry date	01. Feb. 2020
Storage	Room Temperature (20 ± 5°C)

6.1.2 Storage

The test item was stored in a tightly closed vessel at room temperature (20 ± 5°C).

6.1.3 Pre-Treatment

A stock solution containing 1545.7 mg/L was prepared. The carbon content was measured in fivefold diluted solutions with and without membrane filtration in order to estimate the amount to be added to the test flasks. The organic carbon concentration in the filtered 747.45 mg/L) and unfiltered solution (705.5 mg/L) was in the same range.

Thus, the organic carbon concentration in the stock solution was 747.45 mg/L, giving an organic carbon content of 48.36 %.

6.2 Positive Control

Aniline (Phenylamine, $C_6H_5NH_2$, CAS-No. 62-53-3) was used as readily biodegradable positive control. A stock solution containing 2103.2 mg/L in deionised water was prepared and its organic carbon content was measured with 1727.2 mg/L, corresponding to an organic carbon content of the positive control of 82.1 %.

6.3 Test System

6.3.1 Specification

Activated sludge from a biologic sewage treatment plant was used as inoculum. The chosen plant is treating mostly domestic sewage.

6.3.2 Source and Pre-Treatment of inoculum

6.3.2.1 Source

The sludge was taken from the activation basin of the ESN (Stadtentsorgung Neustadt) sewage treatment plant, Im Altenschemel, NW-Lachen-Speyerdorf.

Date of collection: 06. Oct. 2017, batch no: 20171006.

6.3.2.2 Pre-Treatment

The sludge was filtrated, washed with tap water (2x), then washed with and re-suspended in test medium. It was then aerated until use. The dry matter was determined as 4520 mg suspended solids/L.

6.4 Instruments and Devices

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

- Data logger for temperature, ebro
- Analytical scales Mettler Toledo XS 205 DU
- Precision scales Mettler Toledo XS 6001S
- Adjustable pipettes with one-way tips Rainin®
- Carbon analyser TOC multi N/C 2100S, Analytik Jena
- Magnetic stirrers
- pH-meter 3310 wtw
- Heating chamber Heratherm OGS 60
- Ultrasonic bath SONOREX RK 510H Bandelin
- Fridge

Usage and, if applicable, calibration of all instruments followed the corresponding SOP in the current edition. Standard laboratory material was also used.

6.5 Test Vessels

2000 mL-SCHOTT-flasks were used as test vessels, 100 mL scrubber flasks as absorbent vessels.

6.6 Chemicals

All chemicals used in the test were “analytical grade” or otherwise proved suitable.

Note: The weights depend on the final volume which was needed in the test. Actual values are stated in the raw data.

6.6.1 Stock Solutions

6.6.1.1 Solution a

Potassium dihydrogen phosphate (KH_2PO_4)	8.5 g
Dipotassium hydrogen phosphate (K_2HPO_4)	21.75 g
Disodiumhydrogen phosphate dihydrate ($Na_2HPO_4*2H_2O$)	33.4 g
Ammonium chloride (NH_4Cl)	0.5 g
H_2O demin.	ad 1000 mL

The pH was 7.4.

6.6.1.2 Solution b

Calcium chloride ($CaCl_2$)	27.5 g
H_2O demin.	ad 1000 mL

6.6.1.3 Solution c

Magnesium sulphate heptahydrate ($MgSO_4*7H_2O$)	22.5 g
H_2O demin.	ad 1000 mL

6.6.1.4 Solution d

Iron(III) chloride hexahydrate ($FeCl_3*6H_2O$)	0.25 g
Di-sodium-ethylene diaminetetraacetate dihydrate ($Na_2EDTA*2H_2O$)	0.4 g
H_2O demin	ad 1000 mL

6.6.2 Test Medium

The medium was freshly prepared (volumes were adapted to final volume needed in the test).

Composition:

Solution a	10 mL
Solution b	1 mL
Solution c	1 mL
Solution d	1 mL
H_2O demin.	ad 1000 mL

6.6.3 Sodium Hydroxide

NaOH, 0.25 M solution, used for trapping of emitted carbon dioxide.

NaOH, 1.5 M solution, used for scrubbing of purified air.

6.6.4 Mercury Chloride

$HgCl_2$, used for poisoning of abiotic flasks.

6.6.5 Barium Hydroxide

$Ba(OH)_2$ solution, used for checking the purified air (saturated solution, 1:3 diluted).

6.6.6 Hydrochloric Acid

HCl, 2 M solution, used for driving off dissolved CO_2 on day 28.

6.6.7 Reference Items for Carbon Determination

$C_8H_5KO_4$ for TC (Batch no. MKBS1485V, p.A., content 99.99 %), Na_2CO_3 (Batch no. BCBP0581V, p.A., content 100.05 %) and $NaHCO_3$ (Batch no. 217256106, p.A., content ≥ 99.5 %) for IC.

7 PERFORMANCE OF THE STUDY

7.1 Preparations

The medium was prepared from the stock solutions. The stock solution of the positive control was prepared and its DOC was measured. The stock solution containing 1 capsule of PRO-SHAR PAINTBALLS in deionised water was prepared and its DOC measured. The inoculum was taken from its source, washed, aerated and the dry matter was determined.

The test vessels were filled with medium and inoculum. Then, all flasks were aerated for 72 hours with purified, CO_2-free, moistened air to purge the system of CO_2.

7.2 Experimental Parameters

Flask volume	1500 mL
Apparatus blanks	2, containing mineral medium only
Blank Controls	2, containing mineral medium and inoculum
Positive control flasks	2, containing positive control, mineral medium and inoculum
Test flasks	2, containing test item, mineral medium and inoculum
Abiotic control	1, containing test item, mineral medium and $HgCl_2$
Toxicity control	1, containing test item, positive control, mineral medium and inoculum
Inoculum concentration:	25.0 mg/L
Temperature	19.4 – 21.5 °C
Duration	28 days

The test was performed with a nominal start concentration of 20 mg organic carbon/L.

The following amounts of test item and positive control were added to the flasks:

Table 7.2-a Amounts of test item and positive control in the flasks

Flask	Positive Control 1	Positive Control 2	Test 1	Test 2	Abiotic Control	Toxicity Control
Amount PRO-SHAR PAINTBALLS in mg / L	--	--	41.2	41.2	41.2	41.2
Amount Aniline in mg / L	24.4	24.4	---	-	--	24.4
organic C (calculated) in mg / L	20.0	20.0	19.9	19.9	19.9	40.0

Note: All calculations are performed with unrounded values. Therefore, re-calculation with rounded values may lead to slightly different results.

7.3 Apparatus

The test vessels were aerated with purified (by activated charcoal), CO_2-scrubbed, moistened air. The scrubbing of carbon dioxide was achieved by bubbling the purified air through a flask containing 1.5 M NaOH. To control the absence of CO_2, the air was then led through a flask containing a solution of $Ba(OH)_2$ before reaching the test vessels.

Magnetic stirrers were used to prevent deposition of inoculum.

The emitted CO_2 was trapped in 0.25 M NaOH. Two scrubbers containing 100 mL each were connected in series to the test vessels. The initial IC value of the 0.25 M NaOH was separately determined in each flask.

7.4 Sampling

From each front scrubber flask, 9 samples were taken in order to determine the emitted CO_2 (on day 0, 2, 4, 7, 10, 14, 18, 24 and 29). The sample volume was 1 mL. The resulting change in the volume of the front flask was considered in the calculation of emitted CO_2 (see also chapter 8.3.1).

On day 28, 5 mL HCl 2 M was added to each test flask in order to drive off dissolved CO_2. On day 29, samples from both scrubber flasks were taken.

7.5 CO_2 Determination

Analyses of the emitted CO_2 were made by IC measurement using the carbon analyser TOC multi N/C 2100S, Analytik Jena. Each sample was measured in duplicate or triplicate, respectively (depending on the variation between the measured values). The carbon analyser was calibrated with freshly prepared reference solutions containing potassium hydrogen phthalate (TC), sodium hydrogen carbonate and sodium carbonate (IC) every month. After every start, quality control samples were measured.

8 FINDINGS

8.1 Tables

8.1.1 IC-Values

In the following tables, the IC values (given in mg/L) which were measured in the samples of the front scrubber flasks are stated.

Table 8.1-a IC values in mg/L of apparatus blanks, blank controls, front scrubber

Day	Apparatus blank 1	Apparatus blank 2	Blank Control 1	Blank Control 2
0	3.20	3.63	3.02	2.90
2	4.60	3.92	16.01	7.81
4	4.25	4.73	22.34	12.98
7	5.32	6.87	29.10	23.19
10	8.24	10.04	38.89	31.24
14	11.22	12.93	48.77	46.71
18	15.14	15.28	50.08	44.89
24	19.38	19.71	60.93	57.02
29	25.80	26.20	71.24	65.37

Table 8.1-b IC values in mg/L of positive control, test flasks, front scrubber

Day	Positive Control 1	Positive Control 2	Test 1	Test 2	Abiotic Control	Toxicity Control
0	4.64	2.80	3.72	2.84	2.79	2.47
2	13.33	10.11	18.16	19.12	17.70	10.11
4	29.31	50.29	49.19	34.92	17.20	76.33
7	225.85	173.55	65.20	128.89	19.14	303.95
10	252.37	251.90	226.32	211.08	20.07	417.26
14	309.85	306.93	306.87	278.10	24.47	540.07
18	317.54	317.56	316.20	281.61	24.84	514.99
24	351.23	344.49	356.37	319.87	30.59	555.93
29	348.97	339.54	352.70	331.83	36.77	539.68

In the following tables, the IC values which were measured in the samples of the back scrubber flasks are stated.

Table 8.1-c IC values in mg/L of blank controls, apparatus blanks, back scrubber

Day	Apparatus blank 1	Apparatus blank 2	Blank Control 1	Blank Control 2
0	3.62	2.78	3.10	2.84
29	4.53	3.87	3.25	4.06

Table 8.1-d IC values in mg/L of positive control, test flasks, back scrubber

Day	Positive Control 1	Positive Control 2	Test 1	Test 2	Abiotic Control	Toxicity Control
0	2.92	3.02	4.67	3.08	3.14	2.84
29	3.28	7.76	5.50	3.65	3.25	5.62

8.1.2 Net IC

For each flask, the net IC was calculated by subtracting the mean IC value of the apparatus blanks of the corresponding sampling date from the remaining IC values. Exception: Values of day 0 do not need to be corrected.

The net IC values are presented in the following table.

Table 8.1-e Net IC-values in mg/L of front scrubber flasks

Day	Blank Control 1	Blank Control 2	Positive Control 1	Positive Control 2	Test 1	Test 2	Abiotic Control	Toxicity Control
0	3.0	2.9	4.6	2.8	3.7	2.8	2.8	2.5
2	15.2	7.0	12.5	9.3	17.3	18.3	16.9	9.3
4	21.3	11.9	28.2	49.2	48.1	33.8	16.1	75.3
7	26.4	20.5	223.2	170.9	62.5	126.2	16.5	301.3
10	33.2	25.5	246.6	246.2	220.6	205.4	14.3	411.5
14	40.1	38.1	301.2	298.3	298.2	269.4	15.8	531.4
18	38.3	33.1	305.7	305.8	304.4	269.8	13.0	503.2
24	44.8	40.9	335.1	328.4	340.2	303.7	14.5	539.8
29	48.7	42.8	326.4	317.0	330.1	309.2	14.2	517.1

Table 8.1-f Net IC-values in mg/L of back scrubber flasks

Day	Blank Control 1	Blank Control 2	Positive Control 1	Positive Control 2	Test 1	Test 2	Abiotic Control	Toxicity Control
0	3.1	2.8	2.9	3.0	4.7	3.1	3.1	2.8
29	2.3	3.1	2.3	6.8	4.5	2.7	2.3	4.6

8.1.3 pH

In the following table, the pH at the end of the test (before addition of HCl) is given:

Table 8.1-g pH in Test flasks on day 28

Day	Blank Control 1	Blank Control 2	Positive Control 1	Positive Control 2	Test 1	Test 2	Abiotic Control	Toxicity Control
28	7.8	7.8	7.3	7.3	7.4	7.4	6.6	7.3

8.2 Equations

8.2.1 Emitted Carbon in mg/L

Emitted carbon in mg/L test solution in the respective vessel at time t was calculated using the following equation:

$$emittC = \frac{(IC(t) - IC(0)) * VolNaOH(t)}{VolTestVessel}$$

with

emittC	emitted carbon in mg/L test solution
IC(t)	net inorganic carbon in mg/L NaOH in the respective vessel at time t
IC(0)	net inorganic carbon in mg/L NaOH in the respective vessel at the start of the test
VolNaOH (t)	remaining volume NaOH in L in the scrubber at time t (Volume at t = 0 (here: 0.1 L) - ∑ (all sample volumes up to time t))
VolTestVessel	test vessel volume in L (here: 1.5)

For day 29, the IC content of both scrubber flasks was taken into account.

Calculation of emitted carbon is necessary for the assessment of validity. The value obtained with this equation is multiplied with 3.667 (44/12) in order to obtain emitted CO_2.

8.2.2 Degradation in %

The percentage biodegradation in the test flasks was calculated from:

$$\%\ \text{degradation} = \frac{\text{emitted C (Test) in mg/L - Mean emitted C (Controls) in mg/L}}{\text{added C in mg/L}} * 100\%$$

Degradation in positive control and toxicity flasks was calculated analogously.

Abiotic degradation was calculated from:

$$\%\ \text{degradation} = \frac{\text{mg/L emitted C (abiotic)}}{\text{added C in mg/L}} * 100\%$$

8.3 Calculation Results

8.3.1 Emitted Carbon in mg/L

In the following table, the calculated emitted carbon (from net IC given in chapter 8.1.2 and equation stated in chapter 8.2.1) is presented.

Table 8.3-a Emitted carbon in mg/L

Day	Blank Control 1	Blank Control 2	Positive Control 1	Positive Control 2	Test 1	Test 2	Abiotic Control	Toxicity Control
2	0.80	0.27	0.52	0.43	0.90	1.02	0.93	0.45
4	1.19	0.59	1.54	3.03	2.90	2.03	0.87	4.76
7	1.51	1.14	14.13	10.87	3.80	7.98	0.88	19.32
10	1.93	1.45	15.49	15.58	13.88	12.96	0.74	26.18
14	2.35	2.23	18.78	18.71	18.65	16.88	0.82	33.50
18	2.21	1.89	18.87	18.99	18.84	16.73	0.64	31.38
24	2.59	2.36	20.49	20.18	20.86	18.66	0.72	33.31
29	2.74	2.46	19.69	19.52	20.01	18.76	0.64	31.68

8.3.2 Degradation Values

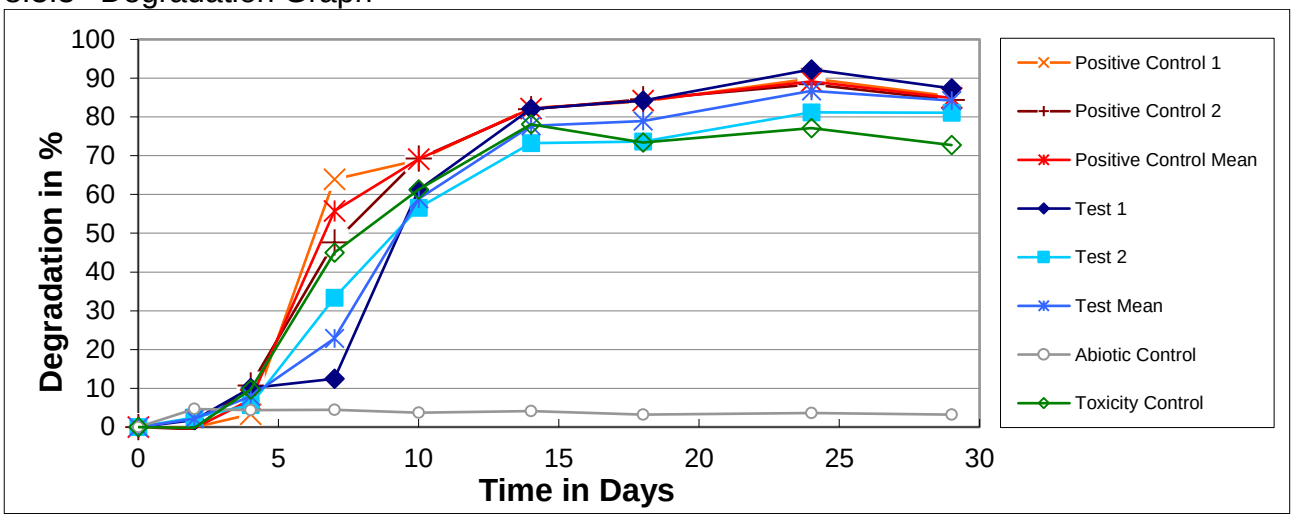
In the following table, the percentage biodegradation is presented:

Table 8.3-b Degradation values in %

Day	Positive Control 1	Positive Control 2	Positive Control Mean	Test 1	Test 2	Test Mean	Abiotic Control	Toxicity Control
2	-0.1	-0.5	-0.3	1.8	2.4	2.1	4.7	-0.2
4	3.3	10.7	7.0	10.1	5.7	7.9	4.4	9.7
7	63.9	47.6	55.8	12.4	33.4	22.9	4.4	45.0
10	68.9	69.3	69.1	61.2	56.6	58.9	3.7	61.3
14	82.3	82.0	82.2	82.1	73.2	77.7	4.1	78.1
18	83.9	84.5	84.2	84.2	73.6	78.9	3.2	73.4
24	89.9	88.4	89.2	92.3	81.2	86.7	3.6	77.2
29	85.3	84.4	84.9	87.3	81.1	84.2	3.2	72.8

Because the values of day 29 are the sum of the IC values in both scrubber flasks, an increase (IC values in flasks B of the test higher than in those of the control) or a decrease (IC values in flasks B of the test lower than in those of the control) of degradation can be observed.

8.3.3 Degradation Graph



9 RESULTS AND VALIDITY

9.1 Results for the Test Item PRO-SHAR PAINTBALLS

- The test item PRO-SHAR PAINTBALLS is considered as "**readily biodegradable**".
- The degree of biodegradation reached 84 % after 28 days.
- The 10-day-window began on day 5, at its end, 78 % degradation were reached, surpassing the pass level of 60 % given in the OECD guideline.
- Abiotic degradation reached 3.2 %.

9.2 Validity

All validity parameters and values are presented in the following table:

Table 9.2-a Validity

Parameter	Criterion	Found	Assessment
IC content of test item solution in medium	≤ 5% of TC	0 %	valid
CO_2 emitted by the controls	< 70 mg/L	9.5 mg/L	valid
Difference within replicates	≤ 20%	6.2%	valid
Degradation of positive control > 60%	≤ 14 days	10 days	valid
Degradation in the toxicity flask on day 14	> 25%	78.1 %	valid

10 DISCUSSION

All validity criteria were met.

Degradation behaviour of positive control and toxicity control was normal. Abiotic degradation reached 3.2 %. Both replicates of the test item showed good correspondence.

If degradation in the toxicity flask is below 25 % after 14 days, the test item can be considered as toxic towards the inoculum. As degradation in the toxicity flask was 78.1 % after 14 days, the test item can be stated as “not toxic towards the inoculum in a concentration of 41.2 mg/L”.

Ready biodegradability is defined in the guidelines as degradation surpassing 60% within 10 days after reaching a level of 10 %.

Because the test item is a mixture, the 10-day window has not to be taken into account. Therefore, regardless of the 10-day window, the test item is considered as “readily biodegradable”.

No observations were made which might cause doubts concerning the validity of the study outcome.

The result of the test can be considered valid.

11 DEVIATIONS

11.1 Deviations from the Study Plan

The following deviation from the study plan was documented:

- Temperature range was 19.4 – 21.5 °C instead of 20.0 – 24.0 °C. As degradation of the positive control was in the normal range, this is considered as uncritical concerning the outcome of the study.

The deviation was assessed and signed by the study director on 10. Nov. 2017.

11.2 Deviations from the Guideline

The following deviation from the guideline was documented:

- Temperature range was 19.4 – 21.5 °C instead of 20.0 – 24.0 °C. As degradation of the positive control was in the normal range, this is considered as uncritical concerning the outcome of the study.

The deviation was assessed and signed by the study director on 10. Nov. 2017.

12 RECORDING AND ARCHIVING

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 15 years. After that, the sponsor’s instructions will be applied (shipment of documentation to sponsor). A retain sample of the test item will be kept in the GLP Substance Archive for 15 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 / toxicological in vitro studies on mammalian cells and bacteria)

Datum der Inspektion / Date of Inspection
(Tag.Monat.Jahr / day.month.year)
13.und 14.10.2014

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

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

Eine erneute behördliche Überprüfung der Einhaltung der GLP-Grundsätze durch die Prüfeinrichtung ist spätestens drei Jahre nach der letzten Inspektion zu beantragen. Ohne diesen Antrag wird die Prüfeinrichtung nach Ablauf der Frist aus dem deutschen GLP-Überwachungsprogramm genommen und diese GLP-Bescheinigung verliert ihre Gültigkeit.

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

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

Verification of the compliance of the test facility with the Principles of the GLP has to be applied for not later than three years after the last inspection. Elapsing this term, the test facility will be taken out of the German GLP-Monitoring Programme and this GLP Certificate becomes invalid.

Landesamt für Umwelt, Wasserwirtschaft und Gewerbeaufsicht 2 Rheinland-Pfalz

Unterschrift, Datum / Signature, Date

8.4.15

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: GLOSSARY

IC	inorganic carbon
DOC	dissolved organic carbon
TOC	total organic carbon
TC	total carbon

15 ANNEX 3: CALIBRATION REPORTS CARBON ANALYSER

Date 12. Oct. 2017 (exemplarily)

aj-Analysensystem multi N/C 2100 S; multiWin 4.03; Geräte-Nr.: N5-108/G

lisa.boeringer

KalibrierReport

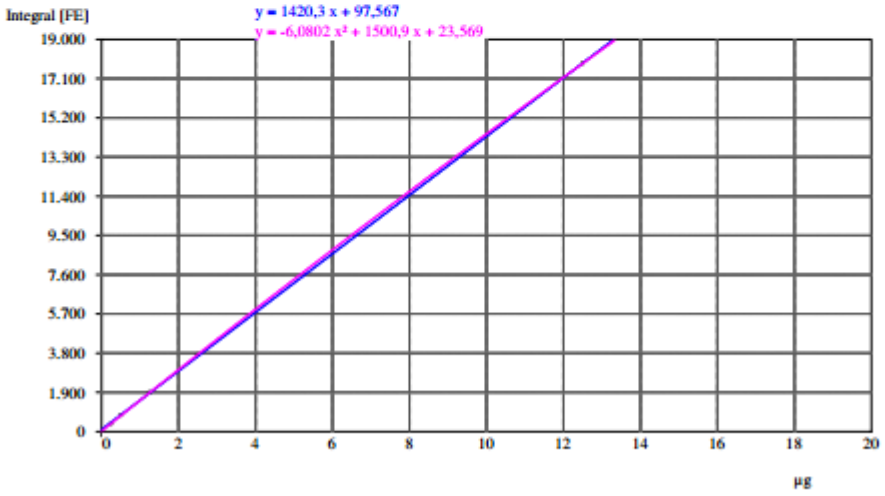
Kalibrierung: Cal_LAUS1_2014_171012_0928
Kalibrierung vom: 12.10.2017 09:28:21 +0200 **Methode:** LAUS1_2014

Benutzer: lisa.boeringer
Kalibrier-Kanal: **IC-500ppm**
Lineare Regression [µg]: c = (k1·I + K0) / V
k0 = -0,06847 k1 = 7,040E-4

Rest-Standardabweichung:	67,917 FE	**Linearität:**	OK
Verfahrensstandardabweichung:	0,47817mg/l	**Varianzhomogenität:**	OK
Verfahrensvariationskoeffizient:	1,4064 %	**Nachweisgrenze:**	901,4µg/l
Korrelationskoeffizient:	0,99997	**Erfassungsgrenze:**	1,80mg/l
Bestimmtheitsmaß:	0,99994	**Bestimmungsgrenze:**	3,61mg/l

Kalibrierung mit konstantem Probenvolumen: 100,00µl

Nr.	Best.	c-soll	m	I-Netto	c-ist	c-ist
H2O	3-3			601,8FE/ml		
1	3-3	2,50mg/l	0,198µg	378,0FE	1,98mg/l	-20,95%
2	3-3	5,00mg/l	0,484µg	784,5FE	4,84mg/l	-3,24%
3	3-3	12,50mg/l	1,28µg	1.910FE	12,76mg/l	2,08%
4	3-3	25,00mg/l	2,55µg	3.726FE	25,55mg/l	2,20%
6	3-3	125,0mg/l	12,49µg	17.835FE	124,9mg/l	-0,10%



aj-Analysensystem multi N/C 2100 S; multiWin 4.03; Geräte-Nr.: N5-108/G

KalibrierReport

Kalibrierung: Cal_LAUS1_2014_171012_0928
Kalibrierung vom: 12.10.2017 09:28:21 +0200 **Methode:** LAUS1_2014

Benutzer: lisa.boeringer
Kalibrier-Kanal: **TC-500ppm**
Lineare Regression [µg]: c = (k1·I + K0) / V
k0 = 0,037935 k1 = 6,919E-4

Rest-Standardabweichung:	61,364 FE	**Linearität:**	OK
Verfahrensstandardabweichung:	0,42461mg/l	**Varianzhomogenität:**	OK
Verfahrensvariationskoeffizient:	0,8846 %	**Nachweisgrenze:**	833,1µg/l
Korrelationskoeffizient:	0,99998	**Erfassungsgrenze:**	1,67mg/l
Bestimmtheitsmaß:	0,99996	**Bestimmungsgrenze:**	3,34mg/l

Kalibrierung mit konstantem Probenvolumen: 100,00µl

Nr.	Best.	c-soll	m	I-Netto	c-ist	c-ist
H2O	3-3			1.859FE/ml		
1	3-3	5,00mg/l	0,456µg	604,1FE	4,56mg/l	-8,81%
2	3-3	10,00mg/l	0,986µg	1.371FE	9,86mg/l	-1,37%
3	3-3	25,00mg/l	2,55µg	3.635FE	25,53mg/l	2,13%
4	3-3	50,00mg/l	5,02µg	7.196FE	50,17mg/l	0,34%
5	3-3	150,0mg/l	14,99µg	21.606FE	149,9mg/l	-0,08%