Final Report

Original 1 of 1

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

Study No.: 21041502G605

Sponsor:
Ropimex R.Opel GmbH
Chemicals, Adexano
Dr. Dirk Kreischer
Bildstocker Str. 12-14
66538 Neunkirchen, Germany

Monitor:
Dr. Dirk Kreischer

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

Study Director:
Elke Klein

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 documents:

- OECD Principles of Good Laboratory Practice and Compliance Monitoring, adopted by Council on 26th November 1997; Environment Directorate, Organisation for Economic Cooperation and Development, Paris 1998 and subsequent advisory/consensus OECD GLP documents (where appropriate).
- 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. In deviation from the guideline, $HgCl_2$ was used in the test, where the retest date was slightly exceeded. This is not critical as the chemical was only used to kill the bacteria and $HgCl_2$ is effective enough to kill even after the retest date.

E. Klein — 15 DEC 2021

Elke Klein, Study Director — Date

Information on Study Organisation:

Study Director	Elke Klein
Deputy Study Director	Norman Ritthaler
Study Plan dated	12. May 2021
Experimental Starting Date	16. Aug. 2021
Experimental Completion Date	22. Sep. 2021

2 QUALITY ASSURANCE UNIT STATEMENT

This study has been inspected by the quality assurance unit according to the principles of Good Laboratory Practice.

All phases of the study (Study plan, performance of the study and Final report) were checked by the quality assurance. Dates of inspections are given below. Findings are reported to the Study Director and Test Facility Management.

The inspection of the performance of short-term studies (duration less than four weeks) may be 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 Final report was written in accordance with the Study Plan and the Standard Operating Procedures of the test facility.

Deviations from the Study plan (if any) were acknowledged and assessed by the Study Director and included in the Final report.

The reported results reflect the raw data of the study.

Phases of Study	Inspected on	Findings reported on	Audit report no.
Study plan	04. May 2021	04. May 2021	210504-01
Performance of study	23. Aug. 2021	23. Aug. 2021	210823-03
Final report	06. Dec. 2021	06. Dec. 2021	211206-01

Schedler

Eva Vogt **on behalf** Dr. Anette Schedler
Quality Assurance

15 DEC 2021
Date

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3 SUMMARY

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

Findings and Results:

The test item Bacoban WB was tested using a concentration of nominally 20 mg organic carbon/L Bacoban WB 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 surpassed the pass level of 60 % after 7 days.

The following data were determined for the test item Bacoban WB:

10-day-window:	day 4 – 14
degradation at the end of 10-day-window	47 %
degradation at the end of the test	65 %
pass level following guideline:	60 % at the end of 10-day-window for pure substances respective 60 % at the end of the test for mixtures

Therefore, when applying the 10-day-window, Bacoban WB is not readily biodegradable following OECD 301B and EU C.4-C respectively.

Because the test item is a mixture the 10-day-window does not have to be taken into account. As degradation reached the pass level of 60% in the course of the test, Bacoban WB is considered as **readily biodegradable.**

4 PURPOSE AND PRINCIPLE OF THE STUDY

This study was performed in order to evaluate aerobic elimination and degradation potential of Bacoban WB in a test for ready biodegradability, using a test item concentration of nominally 20 mg organic carbon/L Bacoban WB.

The test item in a mineral medium was inoculated and incubated under aerobic conditions in the dark. The amount of organic carbon 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 Guideline for the Testing of Chemicals, Part 301 B, adopted 17. Jul. 1992 "CO_2-Evolution-Test (Modified STURM Test)"
- Commission 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 edition 13, valid from 16. Aug. 2021 „Abbaubarkeitstest nach OECD 301B / EU C.4-C"

6 MATERIALS AND METHODS

6.1 Test Item

Designation in Test Facility:	21041502G
Date of Receipt:	15. Apr. 2021
Condition at Receipt:	Ambient 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	Bacoban WB
Batch no.	2101050A - 20210105_WB_imi
CAS no.	not available
Composition	2,2'-Oxydiethanol 28.75 g/100g; Alkyl dimethyl benzyl ammonium chloride 26.00 g/100g; pyridine-2-thiol 1-oxide, sodium salt 2.5 g/100g; Inorganic-organic Polymer based on EINECS listed silanes for long term stability 5.622 g/100g; Cocktail (Fragrance) 1.000 g/100g; water ad 100
Storage	room temperature (20 ± 5 °C)
Expiry date	31. Jan. 2023
Stability	stable under storage conditions
Appearance	clear, yellowish liquid
Purity	not applicable, mixture
Homogeneity	Homogeneous
Production date	05. Jan. 2021
EC no.	unknown
Molecular formula	unknown
Molecular weight	unknown
Vapour pressure	not stated
Solubility in solvents	H_2O: > 1 g/L; EtOH: unknown; acetone: unknown; CH_3CN: unknown; DMSO: unknown; methanol: not stated
Stability in solvents	H_2O: 96 h; EtOH: unknown; acetone: unknown; CH_3CN: unknown; DMSO: unknown; methanol: not stated

6.1.2 Storage in Test Facility

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 1.000 g/L in deionised water was prepared. Its organic carbon was determined in order to estimate the amount to be added to the test flasks.

The TOC was 349.33 mg/L, giving an organic carbon content of 34.93 %.

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 2.1004 g/L in deionised water was prepared and its organic carbon content was measured with 1497 mg/L, corresponding to an organic carbon content of the positive control of 71.27 %.

6.3 Test System

6.3.1 Specification

Activated sludge from a biologic sewage treatment plant was used as inoculum. The chosen plant treats 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 sewage treatment plant, In den Seewiesen, 67482 Edenkoben.

Date of collection: 20. Aug. 2021, batch no: E20210820.

6.3.2.2 Pre-Treatment

The sludge was filtrated through a cloth, washed with test medium (2x) and resuspended in test medium. It was then aerated until use. The dry matter was determined to contain 3.96 g of 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
- Analytical scales Mettler Toledo XSR205DU
- Precision scales Sartorius CPA8201
- Adjustable pipettes with one-way tips, Mettler Toledo
- Carbon analyser TOC multi N/C 2100S, Analytik Jena
- Magnetic stirrers
- pH-meter 3310, wtw
- Drying chamber Heraeus
- 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 with purity >97.5%.

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 dihydrate ($CaCl_2*2\ H_2O$)	36.4 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. 10 mL of solution a were mixed with 800 mL H_2O demin, then 1 mL of solutions b, c and d were added and filled up to 1 L with H_2O demin (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 Instrument TOC multi N/C 2100S

Identification:	114 00 530#A1
Components:	TOC multi N/C 2100S
Manufacturer:	Analytik Jena AG
Software:	multiWin, Version 4.09.05.0005

6.6.8 Quality Control Samples

Date	IC
17. Aug. 2021	1.05382
18. Aug. 2021	1.06103
19. Aug. 2021	1.04355
20. Aug. 2021	1.02506
23. Aug. 2021	1.03253
24. Aug. 2021	1.03620
25. Aug. 2021	1.04614
27. Aug. 2021	1.00936
30. Aug. 2021.	1.04125
01. Sep. 2021.	1.01885
02. Sep. 2021.	1.01806
03. Sep. 2021	1.01978
06. Sep. 2021	1.01186
09. Sep. 2021	1.01374
10. Sep. 2021	1.02276
13. Sep. 2021	1.03849
14. Sep. 2021	1.01600
15. Sep. 2021	1.00313
16. Sep. 2021	0.99469
17. Sep. 2021	1.00896
20. Sep. 2021	1.00786
21. Sep. 2021	0.97020
22. Sep. 2021	0.98575

6.6.9 Reference Items for Carbon Determination

Potassium Hydrogen Phthalate, $C_8H_5KO_4$ for TC
- Batch No.: MKBZ5705V
- Supplier: Sigma Aldrich
- Content: 100.00 %
- CAS-Nr.: 877-24-7
- Expiry Date: 27. Mar. 2023
- Quality Management System: not stated

Sodium Carbonate, Na_2CO_3 for IC
- Batch No.: 247259189
- Supplier: Roth
- Content: 99.8 %, p. A.
- CAS-Nr.: 497-19-8

- Expiry Date: 30. May 2021
- Quality Management System: ISO 9001 Quality Management System

Sodium Hydrogen Carbonate, $NaHCO_3$ for IC
- Producer: Carl Roth
- Expiry date: 05. Feb. 2025
- Batch Nr. 071306105
- Content ≥ 99.5 % p. A.
- CAS-Nr.: 144-55-8
- Quality Management System: ISO 9001 Quality Management System

6.6.10 Calibration

The concentration of IC and TC in the reference solutions was determined as follows:

Linear calibration curve:

$$c_{measured}\ [mg/L] = \frac{k1 * I + k0}{V}$$

with

Calibration from 06. May 2021

for IC high:
k1 = slope ($6.400*10^{-4}$)
I = measured area unit per mL
k0 = intercept (0.084059)
V = sample volume (100 µL)

for IC low:
k1 = slope ($6.773*10^{-4}$)
I = measured area unit per mL
k0 = intercept (-0.010792)
V = sample volume (100 µL)

for TC high:
k1 = slope ($6.378*10^{-4}$)
I = measured area unit per mL
k0 = intercept (0.22644)
V = sample volume (100 µL)

for TC low:
k1 = slope ($6.717*10^{-4}$)
I = measured area unit per mL
k0 = intercept ($4.716*10^{-3}$)
V = sample volume (100 µL)

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 organic carbon was measured. The stock solution of the test item was prepared and its organic carbon was 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	19.7 – 21.9 °C without direct lighting
Duration	28 days

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

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 Bacoban WB in mg / L	--	--	57.3	57.3	57.3	57.3
Amount Aniline in mg / L	28.0	28.0	--	--	--	28.0
organic C (calculated) in mg / L	20.0	20.0	20.0	20.0	20.0	40.0

The added amount of test item and positive control is based on the TOC content (total organic carbon. The TOC content of the water-soluble positive control and the water-soluble test item was determined by TOC measurement, each of a stock solution.
Note: All calculations are performed with unrounded values. Therefore, recalculation 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.

7.4 Sampling

From each front scrubber flask, 9 samples were taken in order to determine the emitted CO_2 (on day, 2, 4, 7, 9, 11, 14, 18, 23 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 0, only one sample of 0,25 M NaOH was sampled. This measured value was used as start value for all treatments.

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). Quality control samples were measured on each measuring day.

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	1.83	1.83	1.83	1.83
2	10.88	10.47	8.63	17.27
4	11.46	15.24	15.78	26.04
7	14.06	20.75	24.16	41.61
9	15.70	17.68	28.39	48.56
11	16.54	17.75	31.26	55.08
14	20.71	17.96	41.76	59.71
18	24.58	18.02	51.88	72.77
23	24.70	23.15	55.90	79.92
29	31.58	31.09	67.35	93.20

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	1.83	1.83	1.83	1.83	1.83	1.83
2	8.67	9.35	15.07	7.39	8.91	8.13
4	42.73	99.63	107.35	19.76	12.21	61.94
7	224.58	196.72	157.98	67.55	13.79	258.00
9	251.60	262.58	163.76	82.20	15.37	306.09
11	269.23	282.73	166.25	96.84	15.66	306.19
14	306.04	284.99	184.24	220.34	15.23	380.89
18	344.82	322.94	228.41	248.74	20.29	472.48
23	347.20	333.76	257.46	263.00	21.12	484.53
29	359.69	350.74	294.50	293.43	24.03	534.30

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	1.83	1.83	1.83	1.83
29	7.47	9.70	6.37	7.82

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	1.83	1.83	1.83	1.83	1.83	1.83
29	6.66	7.17	11.41	6.74	6.27	8.18

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 value of day 0 of the apparatus blank was subtracted from the apparatus blanks of the corresponding sampling dates in advance.

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	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
2	-0.2	8.4	-0.2	0.5	6.2	-1.5	0.1	-0.7
4	4.3	14.5	31.2	88.1	95.8	8.2	0.7	50.4
7	8.6	26.0	209.0	181.1	142.4	52.0	-1.8	242.4
9	13.5	33.7	236.7	247.7	148.9	67.3	0.5	291.2
11	15.9	39.8	253.9	267.4	150.9	81.5	0.3	290.9
14	24.3	42.2	288.5	267.5	166.7	202.8	-2.3	363.4
18	32.4	53.3	325.4	303.5	208.9	229.3	0.8	453.0
23	33.8	57.8	325.1	311.7	235.4	240.9	-1.0	462.4
29	37.9	63.7	330.2	321.2	265.0	263.9	-5.5	504.8

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	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
29	-0.4	1.1	-0.1	0.4	4.7	0.0	-0.5	1.4

Negative values occur, when the apparatus blank was higher than the respective treatment. As the measured values in these blanks as well as in the abiotic control are very low, measurement uncertainties lead to negative degradation values in the abiotic control.

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	7.4	7.3	7.3	7.2	7.3	7.4	6.9	7.6

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:

$$\text{emittC} = \frac{\big(IC(t) - IC(0)\big) * VolNaOH(t)}{Vol\_Testvessel}$$

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))
Vol_Testvessel	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:

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

Degradation in positive control and toxicity flasks was calculated analogously.

Abiotic degradation was calculated from:

$$\%\ \text{degradation} = \frac{emittedC(abiotic)in\ mg/L}{addedC\ in\ mg/L} * 100\%$$

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	Toxi-city Control
2	-0.13	0.44	-0.13	-0.09	0.29	-0.22	-0.12	-0.17
4	0.16	0.83	1.92	5.64	6.14	0.42	-0.07	3.18
7	0.44	1.56	13.40	11.60	9.09	3.24	-0.23	15.56
9	0.75	2.04	15.03	15.74	9.41	4.19	-0.08	18.52
11	0.89	2.40	15.97	16.82	9.44	5.05	-0.09	18.31
14	1.41	2.53	17.97	16.65	10.33	12.60	-0.26	22.66
18	1.90	3.19	20.06	18.70	12.84	14.10	-0.06	27.97
23	1.96	3.43	19.83	19.00	14.32	14.66	-0.17	28.25
29	2.19	3.75	19.92	19.38	15.97	15.90	-0.44	30.51

8.3.2 Degradation Values

In the following table, the percentage biodegradation is presented:

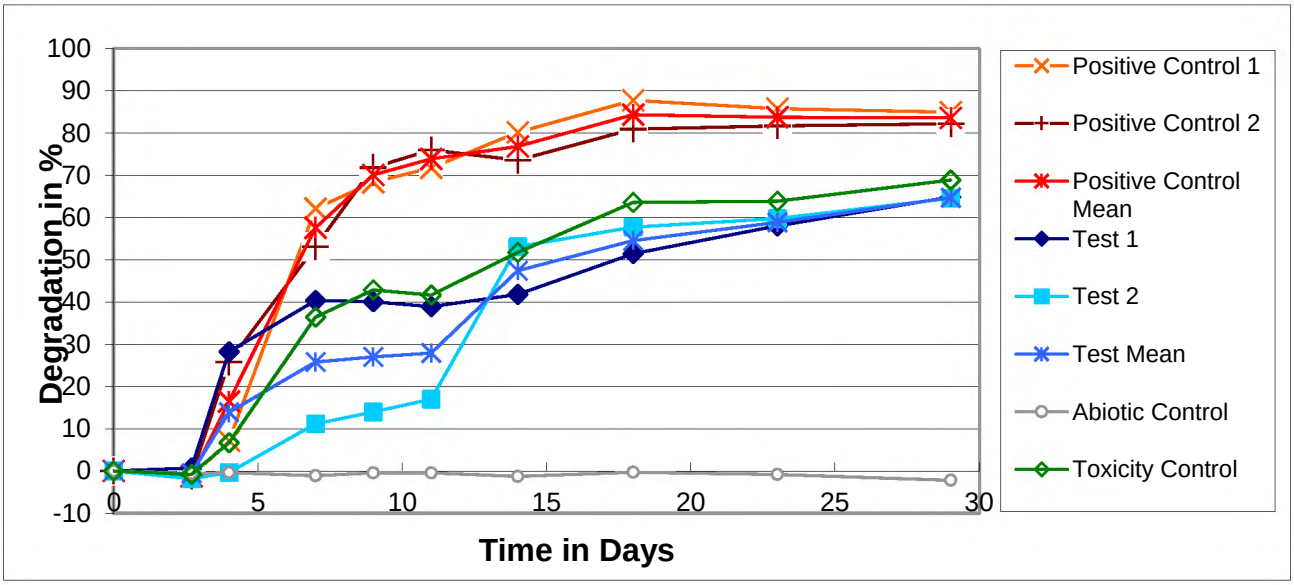
Table 8.3-b Degradation values in %

Day	Posi-tive Control 1	Posi-tive Control 2	Posi-tive Control Mean	Test 1	Test 2	Test Mean	Abiotic Control	Toxi-city Control
2	-1.4	-1.2	-1.3	0.7	-1.8	-0.6	-0.6	-0.8
4	7.1	25.8	16.5	28.2	-0.4	13.9	-0.4	6.7
7	62.1	53.1	57.6	40.4	11.2	25.8	-1.2	36.4
9	68.3	71.9	70.1	40.0	14.0	27.0	-0.4	42.8
11	71.7	76.0	73.9	38.9	17.0	27.9	-0.5	41.7
14	80.2	73.5	76.9	41.8	53.1	47.4	-1.3	51.7
18	87.7	81.0	84.4	51.4	57.7	54.6	-0.3	63.6
23	85.8	81.7	83.8	58.0	59.7	58.9	-0.9	63.9
29	84.9	82.2	83.6	64.9	64.6	64.7	-2.2	68.9

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.

As the measured IC values in the abiotic control are very low, measurement uncertainties lead to negative degradation values while in fact no degradation has taken place.

8.3.3 Degradation Graph



9 RESULTS AND VALIDITY

9.1 Results for the Test Item Bacoban WB

- The test item Bacoban WB is considered as "**readily biodegradable**".
- The degree of biodegradation reached 65 % after 28 days.
- The 10-day-window began on day 4, at its end, 47 % degradation were reached, missing the pass level of 60 % given in the OECD guideline.
- Because the test item is a mixture, the 10-day window has not to be taken into account. As degradation reached the pass level of 60% in the course of the test, Bacoban WB is considered as **readily biodegradable**, within 28 days.
- Abiotic degradation was not observed.

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	10.9 mg/L	valid
Difference within replicates	< 20%	0.3 %	valid
Degradation of positive control ≥ 60%	≤ 14 days	7 days	valid
Degradation in the toxicity flask on day 14	> 25%	51.7 %	non - toxic

10 DISCUSSION

All validity criteria were met.

Degradation behaviour of positive control and toxicity control was normal. Abiotic degradation was not observed. Both replicates of the test item showed very 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 51.7 % after 14 days, the test item can be stated as “not toxic towards the inoculum in a concentration of 57.3 mg/L”.

For pure substances ready biodegradability is defined in the guidelines as degradation reaching the pass level of 60 % within 10 days after reaching a level of 10 %.

The 10-day-window began on day 4, at its end, 47 % degradation were reached, missing the pass level of 60 % given in the OECD guideline.

Because the test item is a mixture, the 10-day window has not to be taken into account. As degradation reached the pass level of 60% in the course of the test, Bacoban WB is considered as **readily biodegradable**, within 28 days.

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 deviations from the study plan were documented:

- Temperature range was 19.7 – 21.9 °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.
- On day 0, only one sample of 0,25 M NaOH was sampled. This measured value was used as start value for all treatments. This is described in the new SOP, because the start value is identical.

These deviations were assessed and signed by the study director on 28. Sep. 2021.

11.2 Deviations from the Guideline

The following deviations from the guideline were documented:

- The flasks for IC measurement were stored in the fridge at 2.1 - 5.6 °C instead of 4 °C. The flasks are closed and CO_2 content cannot change. This is considered as uncritical concerning the outcome of the study.
- The samples were kept in the fridge for more than 48 hours before measurement. This change not the results, therefore this can be considered as uncritical.

These deviations were assessed and signed by the study director on 28. Sep. 2021.

11.3 Deviations from the SOP

The following deviation from the SOP was documented:

- The samples were kept in the fridge for more than 48 hours before measurement. The flasks are closed and CO_2 content cannot change. This is considered as uncritical concerning the outcome of the study.

The deviation was assessed and signed by the study director on 28. Sep. 2021.

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 (destruction of documentation). A retain sample of the test item will be kept in the GLP-Substance Archive for 15 years and then discarded.

Number of originals of the final report to be sent to the sponsor: 0 (pdf file only)

13 ANNEX 1: COPY OF GLP-CERTIFICATE

RheinlandPfalz
LANDESAMT FÜR UMWELT

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)
28. und 29.04.2021

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 confirmed, 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 Rheinland-Pfalz

Unterschrift, Datum / Signature, Date Mainz, 21.06.21

Sabine Riewenherm - Präsidentin -
(Name und Funktion der verantwortlichen Person / name and function of responsible person)

Landesamt für Umwelt
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
TOC	total organic carbon
TC	total carbon