Study Report

Determination of the aerobic ready biodegradability of MC 6.12.87.3, MC ECO, BIO GREASE 400 (three synonim names) in the CO_2 Evolution Test following OECD 301B resp. EU C.4.C

Study No.: 16030401N605

Sponsor:
smazka.ru LLC
Promyshlennaya 40A
198095 St. Petersburg
Russian Federation

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

Study Director:
Manfred Muckle

23. MAI 2015

Date Signature

Second Sponsor:
VMPAUTO LLC, 198095,
Promyshlennaya 40A,
St.Petersburg,
Russian Federation.

Third Sponsor:
Promante SA,
Gruuss-Stroos 53/A17,
L-9991 Weiswampach,
Luxembourg.

Table of Contents

1 MATERIALS AND METHODS

1.1 Test Substance

1.1.1 Specification

The following information of the test substance was provided by the sponsor.

Name	MC 6.12.87.3, MC ECO, BIO GREASE 400 (three synonim names)
Batch no.	#5
Expiry date	Feb. 2017
Storage	Room Temperature: (15.2 – 20.4 °C)

1.1.2 Pre-Treatment

The carbon content of 75.47 % was determined by elemental analysis.

To achieve a maximum bioavailability on the surface of the test vessels, the nominal load of the test item was added into glass beakers and solved in a minimum amount of acetone. This solution was transferred in 2000 mL-SCHOTT-flasks. The glass beaker was rinsed twice with 5 mL acetone to guarantee complete transfer of test item into the test vessel. Then acetone was evaporated completely using compressed air. Thus a thin layer of test item on the surface of the test vessels was archived.

1.2 Positive Control

Aniline (Phenylamine, $C_6H_5NH_2$, CAS-No. 62-53-3) was used as readily bio-degradable positive control. A stock solution containing 2103.4 mg/L in deionised water was prepared and its organic carbon content was measured with 1694.9 mg/L, corresponding to an organic carbon content of the positive control of 80.6 %.

1.3 Test System

1.3.1 Specification

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

1.3.2 Source and Pre-Treatment

1.3.2.1 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: 01. Apr. 2016, batch no: 20160401.

1.3.2.2 Pre-Treatment

The sludge was filtrated, washed with tap water twice, then washed with and re-suspended in test medium. It was then aerated for ≥ 12 hours. The dry matter was determined with 3340 mg suspended solids/L.

1.4 Chemicals

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

1.4.1 Stock solutions

1.4.1.1 Solution a

Potassium dihydrogenephosphate (KH_2PO_4)	8.5 g
Di-potassium hydrogenephosphate (K_2HPO_4)	21.75 g
Di-sodiumhydrogenephosphate dihydrate ($Na_2HPO_4*2H_2O$)	33.4 g
Ammonia chloride (NH_4Cl)	0.5 g
H_2O demin	ad 1000 mL

The pH was 7.4

1.4.1.2 Solution b

Calcium chloride dihydrate ($CaCl_2*2H_2O$)	36.4 g
H_2O demin	ad 1000 mL

1.4.1.3 Solution c

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

1.4.1.4 Solution d

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

1.4.2 Test Medium

The medium was freshly prepared.

Composition:

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

1.4.3 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.

1.4.4 Mercury Chloride

$HgCl_2$, used for poisoning of abiotic flasks

1.1.1 Barium hydroxide

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

1.4.5 Hydrochloric Acid

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

1.4.6 Reference Items for Carbon Determination
Potassium hydrogenphthalate for TC, Na_2CO_3 and $NaHCO_3$ for IC.

1.5 Test Vessels

All glassware was cleaned with the laboratory cleaning agent and then rinsed with tap water (thrice), diluted HCL (once), tap water (thrice) and deionised water (thrice).

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

2 PERFORMANCE OF THE STUDY

2.1 Preparations

The medium was prepared from the stock solutions. 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.

On the day of the start of the test, CO_2-free medium and inoculum was filled into the test flask.

2.2 Experimental Parameters

Flask volume	1500 mL
Apparatus blanks	2, containing mineral medium only
Controls	2, containing mineral medium and inoculum
Positive control flasks	2, containing positive control, mineral medium and inoculum
Test flasks	2, containing test substance, mineral medium and inoculum
Abiotic control	1, containing test substance, mineral medium and $HgCl_2$
Toxicity control	1, containing test substance, positive control, mineral medium and inoculum
Inoculum concentration:	25.0 mg/L
Temperature	19.5 – 21.1 °C
Duration	28 days

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

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

Table 2.2-a Amounts of test substance and positive control in the flasks

Flask	Positive Control 1	Positive Control 2	Test 1	Test 2	Abiotic Control	Toxicity Control
Amount Test Item in mg / L	--	--	26.5	26.5	26.5	26.5
Amount Aniline in mg / L	24.8	24.8	--	--	--	24.8
organic C (calculated) in mg / L	20.0	20.0	20.0	20.0	20.0	40.0

2.3 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.

2.4 Sampling

From each front scrubber flask, 10 samples were taken in order to determine the emitted CO_2. (on days 0, 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 3.2.1).

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

2.5 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 at least in duplicate. The carbon analyser was calibrated with freshly prepared reference solutions containing potassium hydrogen phthalate and sodium carbonate once every month. After every start, quality control samples were measured.

3 FINDINGS

3.1 Tables

3.1.1 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 3.1-a IC Values in mg/L Apparatus Blanks, Controls, front scrubber

Day	Apparatus blank 1	Apparatus blank 2	Control 1	Control 2
0	2.13	1.93	1.91	2.79
2	4.69	4.30	20.91	9.22
4	6.93	5.39	31.27	15.77
7	8.45	8.21	43.00	35.53
9	9.39	9.72	51.89	50.37
11	10.63	11.06	60.01	56.20
14	11.39	12.84	66.00	67.29
18	13.53	15.44	75.29	76.91
23	16.93	18.95	83.46	84.70
29	24.65	29.45	99.55	96.61

Table 3.1-b IC Values in mg/L Positive Control, Test Flasks, front scrubber

Day	Positive Control 1	Positive Control 2	Test 1	Test 2	Abiotic Control	Toxicity Control
0	2.22	2.03	2.20	1.79	2.27	2.39
2	13.75	12.53	50.08	56.33	26.18	36.72
4	85.18	70.69	127.13	151.48	27.95	161.19
7	222.83	211.14	220.69	304.12	30.90	357.59
9	262.55	260.12	287.07	355.72	30.80	417.70
11	300.51	302.82	322.81	401.44	32.31	469.35
14	315.19	314.51	345.73	421.32	32.04	514.63
18	334.79	343.64	383.36	457.66	35.67	569.49
23	336.21	346.13	391.53	465.21	37.41	581.27
29	377.79	382.56	436.62	533.02	45.62	653.37

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

Table 3.1-c IC Values in mg/L Controls, Apparatus Blanks, back scrubber

Day	Apparatus blank 1	Apparatus blank 2	Control 1	Control 2
0	2.29	2.36	2.26	2.44
29	7.91	3.53	3.90	6.65

Table 3.1-d IC Values in mg/L Positive Control, Test Flasks, back scrubber

Day	Positive Control 1	Positive Control 2	Test 1	Test 2	Abiotic Control	Toxicity Control
0	2.42	1.95	2.20	2.19	2.45	2.50
29	6.00	5.62	4.49	8.49	4.30	7.02

3.1.2 Net IC

For each flask, the IC value which was measured at the start of the test (d = 0) was subtracted from all following measurements. The net IC was calculated using this corrected measurement value and subtracting the mean IC value of the apparatus blanks of that sampling date.

The net IC values are presented in the following table.

Table 3.1-e Net IC-values in mg/L front scrubber flasks

Day	Control 1	Control 2	Positive Control 1	Positive Control 2	Test 1	Test 2	Abiotic Control	Toxicity Control
0	1.9	2.8	2.2	2.0	2.2	1.8	2.3	2.4
2	18.4	6.8	11.3	10.1	47.6	53.9	23.7	34.3
4	27.1	11.6	81.1	66.6	123.0	147.4	23.8	157.1
7	36.7	29.2	216.5	204.8	214.4	297.8	24.6	351.3
9	44.4	42.8	255.0	252.6	279.5	348.2	23.3	410.2
11	51.2	47.4	291.7	294.0	314.0	392.6	23.5	460.5
14	55.9	57.2	305.1	304.4	335.6	411.2	22.0	504.5
18	62.8	64.5	322.3	331.2	370.9	445.2	23.2	557.0
23	67.6	68.8	320.3	330.2	375.6	449.3	21.5	565.4
29	74.5	71.6	352.8	357.5	411.6	508.0	20.6	628.4

Table 3.1-f Net IC-values in mg/L back scrubber flasks

Day	Control 1	Control 2	Positive Control 1	Positive Control 2	Test 1	Test 2	Abiotic Control	Toxicity Control
0	2.3	2.4	2.4	2.0	2.2	2.2	2.5	2.5
29	0.5	3.3	2.6	2.2	1.1	5.1	0.9	3.6

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

Table 3.1-g pH Test flasks on day 28

Day	Control 1	Control 2	Positive Control 1	Positive Control 2	Test 1	Test 2	Abiotic Control	Toxicity Control
28	7.3	7.3	7.2	7.2	7.4	7.4	6.8	7.3

3.2 Equations

3.2.1 Emitted Carbon in mg/L
Emitted Carbon in mg/L test solution at time t is 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)	inorganic carbon in mg/L NaOH at time t
IC(0)	inorganic carbon in mg/L NaOH 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.

3.2.2 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:

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

3.3 Calculation Results

3.3.1 Emitted Carbon in mg/L

In the following table, the calculated emitted carbon (from IC values given in 3.1 and equation stated in 3.2.1) is presented.

Table 3.3-a Emitted carbon in mg/L

Day	Control 1	Control 2	Positive Control 1	Positive Control 2	Test 1	Test 2	Abiotic Control	Toxicity Control
2	1.09	0.26	0.60	0.53	3.00	3.44	1.42	2.10
4	1.65	0.58	5.15	4.22	7.89	9.51	1.41	10.11
7	2.25	1.71	13.86	13.12	13.72	15.20	1.44	22.56
9	2.72	2.56	16.18	16.04	16.83	17.55	1.34	26.10
11	3.12	2.82	18.33	18.49	18.71	19.54	1.34	29.02
14	3.38	3.41	18.98	18.95	19.78	20.20	1.23	31.47
18	3.78	3.82	19.85	20.41	21.63	21.58	1.30	34.39
23	4.03	4.05	19.51	20.13	21.66	21.48	1.18	34.53
29	4.29	4.23	21.28	21.59	23.40	24.15	1.01	38.05

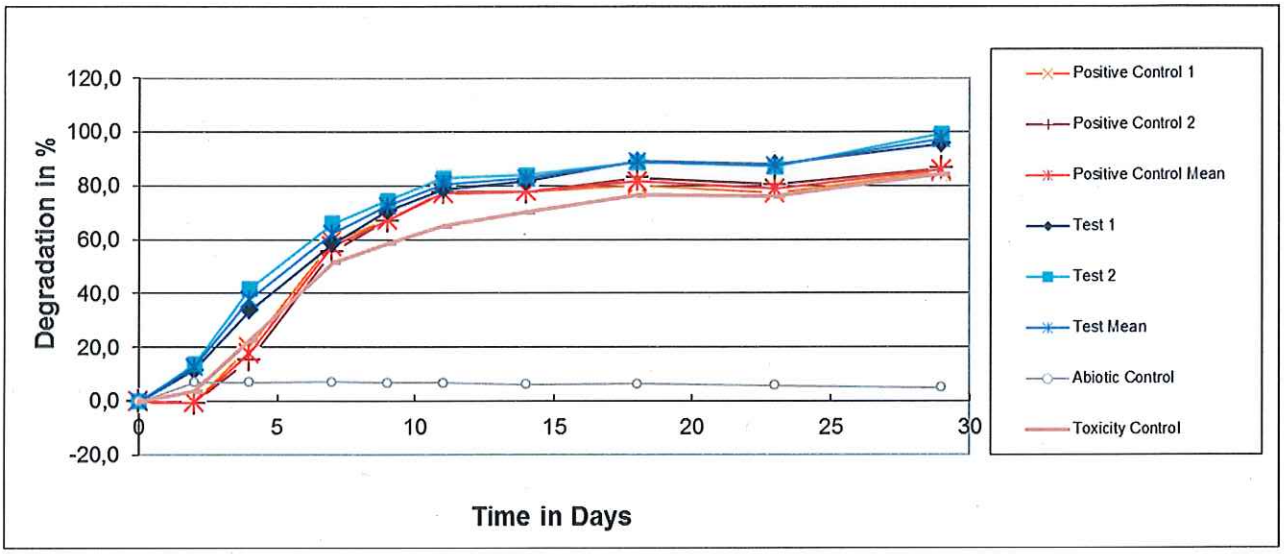
3.3.2 Degradation Values

In the following table the percentage biodegradation is presented:

Table 3.3-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	-0.4	-0.7	-0.6	11.6	13.8	12.7	7.1	3.6
4	20.2	15.5	17.8	33.9	41.9	37.9	7.0	22.5
7	59.4	55.7	57.5	58.6	66.0	62.3	7.2	51.4
9	67.7	67.0	67.3	70.8	74.5	72.7	6.7	58.6
11	76.8	77.6	77.2	78.6	82.7	80.7	6.7	65.1
14	77.9	77.8	77.8	81.8	83.9	82.9	6.2	70.1
18	80.2	83.0	81.6	89.0	88.8	88.9	6.5	76.4
23	77.4	80.5	78.9	88.0	87.1	87.6	5.9	76.2
29	85.1	86.6	85.9	95.6	99.3	97.5	5.0	84.4

3.3.3 Degradation Graph



4 RESULTS AND VALIDITY

4.1 Results for the Test substance MC 6.12.87.3, MC ECO, BIO GREASE 400 (three synonim names)

- The test substance MC 6.12.87.3, MC ECO, BIO GREASE 400 (three synonim names) is considered as "**readily biodegradable**".
- The pass level for ready biodegradability is 60% based on ThCO2 production.
- The degree of biodegradation reached 98 % after 28 days.
- The 10-day-window began on day 12, at its end, 81 % were reached, surpassing 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. Nevertheless, the test item is considered as "readily biodegradable".
- The abiotic degradation reached 5 %.

4.2 Validity

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

Table 4.2-a Validity

Parameter	Criterion	Found	Assessment
IC content of test item solution in medium	≤ 5% of TC	13.22 %	*see below
CO_2 emitted by the controls	< 70 mg/L	15.6 mg/L	valid
Difference within replicates	≤ 20%	3.8 %	valid
Degradation of positive control > 60%	< 14 days	9 days	valid
Degradation in the toxicity flask on day 14	> 25%	70 %	valid

*The IC content of test item in medium was more than 5 % of TC as demanded in the guideline. This is due to the fact that the test item is very poorly soluble in water. Therefore the validity criterion is not applicable for the test item.

5 ANNEX: GLOSSARY

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