DECAPFORT

ZAHN-WELLENS / EMPA TEST
OECD 302 B
Commission Regulation (EC) No 440/2008

Report

BMG study no. A14-02322/b

December 2014

BMG
AN ARCADIS COMPANY

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1 Preface

1.1 General

Title	DECAPFORT ZAHN-WELLENS / EMPA TEST OECD 302 B Commission Regulation (EC) No 440/2008
Sponsor	Menegalli SA Avenue de Provence 10 CH - 1007 Lausanne
Study Monitor	Mr. Orlando Menegalli
Test Facility	BMG Engineering Ltd. Ifangstrasse 11 CH-8952 Schlieren

1.2 Responsibilities

Study Director	Dr. Philippe Matter

1.3 Schedule

Experimental starting date	6 November 2014
Experimental completion date	4 December 2014

1.4 Archiving

Raw data and a copy of the final report are stored in the archives of BMG Engineering Ltd. in Schlieren for 5 years.

1.5 Test Guidelines

The study procedures described in this study plan meet the requirements of the following test guidelines:

- Organisation for Economic Cooperation and Development. OECD Guidelines for the Testing of Chemicals - Inherent Biodegradability: Zahn-Wellens / EMPA Test, TG 302 B, adopted 17th July 1992.
- Commission Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), C.9. Zahn-Wellens Test. (O.J. L 142 of 31.5.2008).
- The test procedure was conducted as described in detail in BMG's standard operating procedure (Standardarbeitsanweisung BMG-1018).

1.6 Signature

BMG Engineering Ltd.
Ifangstrasse 11
CH-8952 Schlieren

BMG study no.	A14-02322/b
Test item	DECAPFORT
Title	DECAPFORT ZAHN-WELLENS / EMPA TEST OECD 302 B Commission Regulation (EC) No 440/2008
Study Director	Dr. Philippe Matter

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Date: 5 Januar 2014

1.7 Quality assurance statement

It is certified that the test for inherent biodegradability of DECAPFORT was carried out under the study director's supervision using the guidelines and standard operating procedures described in this report and that this report forms a true and accurate record of the procedures performed and of the results obtained.

The study was conducted in accordance with ISO/IEC 17025

2 Summary

The biodegradability of DECAPFORT exposed to activated sludge of a municipal sewage treatment plant was investigated under aerobic static conditions.

The biodegradation of DECAPFORT based on individual DOC determinations reached 99% after 28 days.

The main degradation process occurred during the first 4 days, when more than 90% of the initial DOC was removed. Maximum biodegradation was attained after 18 days of incubation. No significant adaptation period (lag phase) was observed.

No significant elimination of the test item due to adsorption to the activated sludge, on the glass surface or other physico-chemical processes was observed.

The positive control, diethylene glycol, showed 99% biodegradation after 14 days of incubation thus confirming suitability of inoculum and test conditions. The test was considered valid, since the degradation of the reference compound reached more than 70% within 14 days of incubation.

The biodegradation of DECAPFORT based on CO_2 evolution and calculated as % $ThCO_2$ reached 71% after 21 days of incubation, showing that partial mineralization of the test item occurred. The extent of mineralization of the procedure control with diethylene glycol was 72%.

Based on these results DECAPFORT can be termed as ultimately biodegradable under the conditions of the OECD guideline no. 302, since more than 70% degradation was attained after 28 days of contact time.

Due to a DOC elimination of more than 90% and a mineralisation of more than 70% of the $ThCO_2$ DECAPFORT can be termed as well biodegradable.

3 Purpose

The objective of this study was to determine the biodegradability of DECAPFORT under aerobic static conditions using the Zahn-Wellens / EMPA Test.

In this method, activated sludge, mineral nutrients and the test material as the sole carbon source are incubated together in a glass vessel placed on an orbital shaker under controlled conditions. The degradation of the test material is monitored by the determination of the dissolved organic carbon (DOC) at regular time intervals. The ratio of eliminated DOC after each interval to the value 3 hours after the start of the test is expressed as percentage biodegradation. The percentage biodegradation was plotted against time to give the biodegradation curve.

The produced CO_2 was determined during the test as an additional measure of the biodegradation expressed as the percentage of the theoretical value (% $ThCO_2$).

4 Materials and methods

4.1 Test system and test conditions

Inoculum	Activated sludge from the aeration tank of the ARA Werdhölzli (CH-8048 Zürich), a municipal biological waste water treatment plant, not adapted, not pre-conditioned; 0.2 g/l dry matter in the final mixture
Sampling	4 November 2014 ; 10.00 a.m.
Test units	1 litre closed glass bottle containing a total volume of test solution of 800 ml; aerated with CO_2-free air and fitted to gas-absorption bottles containing 125 ml of 0.13 M KOH
Incubation	Temperature-controlled dark room
Temperature	22 ± 2 °C
Test medium	Aerobic mineral salts medium (Table 1) prepared with de-ionized water (conductivity: <1.5 µS/cm; DOC: <0.3 mg/l)
Feed	None, DECAPFORT or reference as sole organic carbon sources
Test duration	28 days

4.2 Test item

Identification	DECAPFORT
BMG sample no.	M1410-11243-01
Test item storage	At room temperature, protected from light
Stability	Stable under storage conditions

4.3 Reference item

Identification	Diethylene glycol
Purity	99% pure
Source	VWR International, 201, rue Carnot, FR-94126 Fontenay-sous-Bois
Batch no.	S4920931 746
Reference item storage	At room temperature protected from light
Stability	Stable under storage conditions

4.4 Test concentrations

Test item	476 mg/l (49.3 mg C/l)
Reference item	111 mg/l (46.4 mg C/l)

4.5 Test procedure

The activated sludge was used after sampling from the treatment plant without adaptation. However, the sludge was pre-conditioned for 2 days (aerated but not fed). Prior to the test the sludge was washed twice with tap water and once with mineral medium. The test material was diluted with mineral salts medium to give a final DOC concentration of about 50 mg/l. The activated sludge was applied to give a dry substance content of 0.2 g/l. The dry substance content of the activated sludge is gravimetrically determined in regular intervals after drying of the sludge at 105°C.

For each test series the following test flasks were set up:

Test suspension (T), 2 replicates:	containing activated sludge, test medium and test item
Blank control (B), 2 replicates:	containing activated sludge and test medium
Procedure control (R), 2 replicates:	containing activated sludge, test medium and diethylene glycol as ready biodegradable reference item

The test vessels were stirred and aerated with synthetic CO_2-free air for a maximum test period of 28 days. The air leaving the individual vessels was passed through gas-absorption bottles filled with KOH. The pH-value was checked at the beginning and was adjusted to pH 7.4 +/- 0.2 with H_2SO_4. At the end of the test the pH was recorded.

The elimination of the test material was followed by DOC determinations at regular intervals. First samples were analyzed before the addition of the sludge and 3 h after starting the test. The trapped CO_2 in the gas-absorption bottles was determined as inorganic carbon (IC).

4.6 Chemical analyses

Dissolved organic carbon (DOC) was determined in duplicate with a Shimadzu TOC-Analyzer TOC-L CSH (Shimadzu Schweiz GmbH, Römerstr. 3, CH-4153 Reinach). For each determination at least 3 single injections were performed.

Aliquots from the individual test vessels were centrifuged (10 min at 4500 g) and the supernatant acidified to pH <2. Prior to analysis the samples were sparged with CO_2-free high purity air for 5 min to remove inorganic carbon.

Inorganic carbon (IC) was determined in samples taken from the gas-absorption bottles. The analysis was performed using the IC mode of the TOC-Analyzer. For each determination at least 3 single injections were performed.

Table 1 Composition of the mineral salt medium

Stock solution A (pH 7.4)	10 ml/l	Ammonium chloride (NH_4Cl) Di-sodium hydrogenphosphate (Na_2HPO_4 x 2 H_2O) Potassium dihydrogenphosphate (KH_2PO_4) Di-potassium monohydrogenphosphate (K_2HPO_4)	0.50 g/l 33.40 g/l 8.50 g/l 21.75 g/l
Stock solution B	1 ml/l	Calcium chloride ($CaCl_2$)	27.50 g/l
Stock solution C	1 ml/l	Magnesium sulfate ($MgSO_4$ x 7 H_2O)	22.50 g/l
Stock solution D	1 ml/l	Iron (III) chloride ($FeCl_3$ x 6 H_2O)	0.25 g/l

5 Evaluation of the test results

5.1 Definitions

The degree of biodegradation/elimination attained at the end of the test after 28 days or, if degradation is attained in less than 28 days, at an earlier time, is reported as "inherent, ultimate biodegradability in the static test (after x days)" if more than 70% biodegradation was reached. Evidence of "inherent, primary biodegradability" is given, if an elimination of the test item between 20 and 70% was reached. According to the revised introduction of the guidelines for the testing of chemicals (OECD) the evaluation is made based on biological/chemical oxygen demand or DOC removal.

While the evaluation based on BOD/COD as well as based on IC gives information on the mineralization of the test item, DOC removal yields only information on the elimination of the test item. Hence, the evaluation whether the item shows "inherent, ultimate biodegradability" or "inherent, primary biodegradability" was made based on the IC data.

Hence, also the degree of biodegradation was calculated as % of $ThCO_2$. The $ThCO_2$ (Theoretical carbon dioxide evolution in mg) is the total quantity of carbon dioxide calculated to be produced from the known or measured carbon content of the test material when fully mineralized.

If the result of analysis of the sample 3 h after the start of the test is significantly different from the initial value, the amount of deficient DOC is reported as "elimination due to adsorption by the activated sludge or other physico-chemical processes".

5.2 Calculation of the percentage biodegradation

The percentage removal of DOC is calculated using the following equation:

$$D_T = [\ 1 - \frac{C_t - C_B}{C_A - C_{BA}}\ ] \times 100$$

D_T is the biodegradation/elimination at time t in %

C_t is the mean DOC value of the test suspension at time t in mg/l

C_B is the mean DOC value of the blanks at time t in mg/l

C_A is the mean DOC value of the test suspension after 3 h in mg/l

C_{BA} is the mean DOC value of the blanks after 3 h in mg/l

The percentage biodegradation/elimination rounded to the nearest full percent is calculated from the DOC values of the last measurement.

The percentage biodegradation from the produced CO_2 is calculated from:

$$\%\ ThCO_2 = \frac{\text{mg IC from test unit - mg IC from inoculum blank}}{\text{mg DOC added as test material}} \times 100$$

$\%\ ThCO_2$ is the biodegradation at time t in % of the theoretical CO_2

IC is the mean inorganic carbon value of the individual absorption bottles at time t in mg/l

DOC is the organic carbon content of the added test material at the beginning of the test

5.3 Validity criteria

The test is considered valid if the procedure control shows the removal of the reference compound by at least 70% within 14 days.

6 Data compilation

The following data were recorded on data sheets and transcribed for compilation and analysis: amount of test and reference material applied, pH determinations.

The following data were recorded on-line (or on data sheets, as appropriate): DOC and IC determinations.

The TOC Analyzer is validated with respect to data collection, storage and retrievability.

No statistical analysis was performed.

7 Results and discussion

7.1 Determination of biodegradation / elimination

The results of the DOC elimination are presented in Table 2 and Figure 1.

The mineralization data are summarised in Table 3.

The biodegradation of DECAPFORT based on individual DOC determinations reached 99% after 28 days.

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7.2 Remarks and alterations of the standard procedure

Due to a measurement artefact at day 28, the evaluation of the mineralisation is based on the 21 day data.

7.3 Validity of the test

All the validity criteria as specified in section 5.3 were met.

Table 2 DOC concentrations of test suspension, blank control, procedure control and calculation of degradation data

Time (days)	Blank control (B) *	Procedure control with diethylene glycol (R) *			Test suspension with test item (T) *		
	DOC (mg/l)	DOC (mg/l)	DOC net. (mg/l)	Degradation (%)	DOC (mg/l)	DOC net. (mg/l)	Degradation (%)
0	0.9	44.9	44.0	-	49.0	48.1	-
0.125	1.1	45.4	44.4	0	49.0	48.0	0
1	1.7	47.4	45.7	-3	47.5	45.7	5
4	2.4	38.9	36.5	18	22.0	19.6	59
7	3.5	3.2	-0.3	101	6.7	3.2	93
11	2.6	2.3	-0.3	101	4.8	2.2	95
14	3.6	4.0	0.4	99	6.3	2.7	94
18	2.8	2.7	-0.1	100	2.8	0.1	100
21	4.2	4.4	0.1	100	4.6	0.4	99
25	3.2	2.9	-0.3	101	4.1	0.9	98
27	2.1	2.3	0.2	100	2.7	0.6	99
28	2.6	2.1	-0.5	101	2.9	0.3	99

* Mean of two replicates

Figure 1 Degradation curve for DECAPFORT

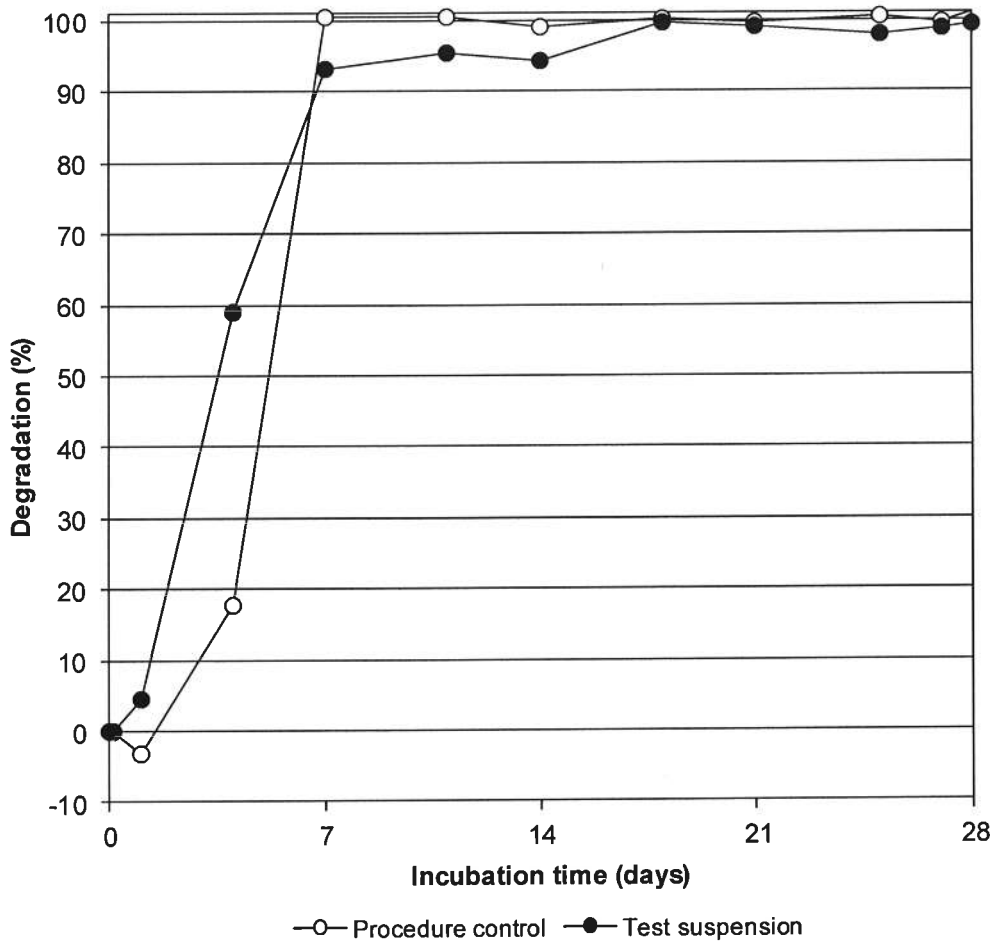

Table 3 IC concentrations, calculated from the concentrations in the gas absorption bottles, of test suspension, inoculum blank and procedure control and corresponding degradation data

Time (days)	Test suspension (mg IC/l)	Procedure control (mg IC/l)	Blank control (mg IC/l)	Biodegradation Test suspension (in % of ThCO2)	Biodegradation Procedure control (in % of ThCO2)
0	nd	nd	nd	-	-
7	49.2	49.0	19.9	60	63
14	63.2	63.4	33.5	60	64
21	69.8	68.4	34.9	71	72
28	66.8	70.3	36.9	*artefact*	72

nd not determined