Final Report S-2017-00234 AM

EVALUATION OF AEROBIC BIODEGRADABILITY ON "#109 BLACK"

Study Program n.: S-2017-00234 AM

Contract n.: M3O820160381-01

Sponsor:
NORIS COLOR GMBH
ZIEGELHUETTENER STR 1
95326 KULMBACH
GERMANY

Test Facility:
EUROFINS BIOLAB SRL
VIA B. BUOZZI, 2
20090 VIMODRONE (MI)
ITALIA

Test item: #109 BLACK

Study Director (Dr. C. Giarei) .....

Released on: March 25th, 2017

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COMPLIANCE WITH GOOD LABORATORY PRACTICE

I the undersigned declare that the studies described in this report have been conducted under my supervision and in compliance with the following standards of Good Laboratory Practice:

- OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring - OECD principles of Good Laboratory Practice (as revised in 1997) – Environment Directorate – Organisation for Economic Co- Operation and Development, Paris 1998.
- Legislative decree n. 50 of March the 2nd, 2007. Enforcement of Community Directives 2004/9/CE e 2004/10/CE, concerning the inspection and verification of Good Laboratory Practice and the drawing of the legislative, regulatory and administrative dispositions relative to the application of Good Laboratory Practice rules, to the control of their application on the assays performed on the chemical substances (GU n.86 of April the 13th, 2007).
- United States Food and Drug Administration, Title 21 Code of Federal Regulations Part 58, Federal Register 22 December 1978, and subsequent amendments.
- Certification N. 038/2013 released by the Italian Ministry of Health on November 19th 2013 and Provisional Certificate released on November 20th 2015 authorizing Eurofins Biolab S.r.l. to perform analyses in compliance with the principles of good laboratory practices (http://www.eurofins.it).

There were no circumstances that may affected the quality or integrity of the study.

Study Director
(C. Giarei)

Date

Eurofins Biolab S.r.l.
Società con Socio unico sottoposta
a direzione e coordinamento della società
Eurofins Pharma Services Italia Holding
parte di Eurofins Scientific Group
InfoFarma@eurofins.com
www.eurofins.it www.biolab.it

Via Bruno Buozzi, 2
20090 Vimodrone (MI)
Tel. + 39-022507151
Fax + 39-0225071599

certificata@pec.biolab.it

C.SOC. € 100.000 i.v.
P. IVA 00762140960
C.F. 03765750157
REA MI 966696
D-U-N-S 429117112
CIT005

QUALITY ASSURANCE STATEMENT

The study was assessed for compliance with the approved study program and the Standard Operating Procedures of Eurofins Biolab Srl.
The study and/or the test facility were periodically inspected by the Quality Assurance unit according to the corresponding SOPs. These inspections and audit were carried out by the Quality Assurance unit, personnel independent of staff involved in the study.

The undersigned hereby certifies the dates on which the inspections have been carried out and reported to the Director of the Study and to Eurofins Biolab's S.r.l. Management:

QAU INSPECTIONS	
PHASE	**DATE**
Experimentation:	
-Audit process-based:	September 13th - 15th, 2016
-Audit study-based:	//
Documentation:	
- Study program	January 30th, 2017
- Raw data	March 25th, 2017
- Final report	March 25th, 2017

This report accurately reflects the raw data.

Marchesi Carlotta
QA GLP
(C. Marchesi)

March 25th, 2017
Date

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Experimentation S-2017-00234 AM – Evaluation of aerobic biodegradability of organic compounds in aqueous medium

ASSAY SYSTEM

Method of mud sampling

Biodegradability of a substance is based on different parameters, and among them composition and concentration of bacterial biomass play an important role.
For the preparation of the inoculum a sample of aerobic sludge has been selected by the mixed treatment plant of urban (about 66%) and industrial (about 34%) liquid sewage situated at San Rocco - Monza (MB), Italia.
The plant of treatment is managed by "BRIANZACQUE SRL (Monza)".

Mud preparation

In the laboratory the sampled muds have been mixed and let settle, keeping them in aerobic conditions.
The mud samples, before their use, have been analysed to check its ability to form colony units.
Then it has been centrifuged, washed and analyzed to quantify the suspended solids concentration for the inoculum preparation. The inoculum was prepared in order to have a concentration of suspended solid of about 4 mg/L.

REAGENTS

Anhydrous potassium dihydrogenphosphate	SIGMA ALDRICH
Anhydrous dipotassium hydrogenphosphate	SIGMA ALDRICH
Disodium hydrogenphosphate dehydrate	SIGMA ALDRICH
Ammonium chloride	SIGMA ALDRICH
Calcium chloride dehydrate	SIGMA ALDRICH
Magnesium sulfate heptahydrate	SIGMA ALDRICH
Iron chloride (III) hexahydrate	SIGMA ALDRICH
Sodium Hydroxide	SIGMA ALDRICH
Deionized water	produced in situ by MilliQ-Biocel A10

EQUIPMENTS AND MATERIALS

Carbon analyzer	SHIMADZU
Magnetic Stirrer	GHIARONI
Multiparametric (pH-meter oxygen meter)	WTW
Hood	ASAL
Centrifuge	REMI
Balance	METTLER
Thermal balance	SHIMADZU
Glass bottles with hermetic seal	GHIARONI
Filtration device	GHIARONI
Micropipettes	GILSON
Refrigerator	WHIRPOOL
High precision syringes	GHIARONI
MilliQ-Biocel A10	MILLIPORE
Common Laboratory equipment	

REFERENCE SUBSTANCE

Reference substance	*Supplier*	*Batch*	*Expiry date*
Sodium Benzoate	Sigma-Aldrich	SLBM8408V	March 2017

Preparation of the reference substance
Considering the Sodium Benzoate reference substance molecular formula, a starting measured concentration of 20.02 mg/l of organic carbon (TOC) has been used.

Blank preparation
Water containing Culture Medium has been used as blank with the addition of the inoculum of the muds.

Test sample + Reference substance
Test sample and reference substance have been put together in order to have a final nominal concentration of 41.11 mg/l of organic carbon (TOC). The results of this set of bottles were not considered because the substance is resulted biodegradable.

Conditions of assay execution
The prepared bottles have been incubated at 25°C ± 2°C for about one hour to equilibrate and release gas excess.
Thereafter, the inoculum has been added replicate bottles, closed and incubation continued under stirring in darkness at 25°C ± 2 °C for 28 days.
At least 12 hours before every determination of inorganic carbon (TIC) the reaction in one bottle of blank, one of reference substance and one of the test item has been stopped with the addition of 6 ml of sodium hydroxide (1 M). The detection of concentration of inorganic carbon (TIC) has been measured weekly.

Determination of total inorganic carbon (TIC)
Determination of total inorganic carbon has been done using an automatic SHIMADZU TOC analyser. It is a high sensitivity instrument based on the combustion catalytic oxidation method (680°C). The carbon dioxide generated by oxidation has been detected using an infrared gas analyzer (NDIR). Samples, properly diluted with deionised water with low TOC contents, have been analysed by means of an integrated sampling system directly from vials containing 40 ml volume.

EXPRESSION OF RESULTS

The calculation of biodegradation is done at every sampling time for the reference substance, sample and for blank.

Total biodegradation will be calculated using the equation:

$$Dt = \frac{(TICt - TICb)}{TOCi} \times 100$$

Where:
D_t = total biodegradation calculated with reference to values of blank, expressed in percentage.

TIC_t = concentration of inorganic carbon, in mg, produced by biodegradation of the test item.

TIC_b = concentration of inorganic carbon, in mg, produced by biodegradation in blank.

TOC_i= concentration of organic carbon of the test item, in mg, added at the beginning.

The result of biodegradability is expressed as the percentage ratio between the value of inorganic carbon measured and the value of organic carbon added at the beginning. The value of inorganic carbon used in the calculations is the highest recorded during the 28 days of the test.

INTERPRETATION OF RESULTS

The test is considered valid, according to ISO 14593:1999 if:
the percentage of average degradation of the reference substance is more than 60% after 14 days of incubation.
According to the OECD GUIDELINE FOR TESTING OF CHEMICALS – 2006. Revised introduction to the OECD guidelines for testing of chemicals, SECTION 3. Part 1, a substance is considered biodegradable when its level of biodegradability is at least 60%, within a period of 28 days.

RESULTS

Quality criteria of the test are satisfied (see table below and Figure N.2).
The mean amount of TIC produced from blank at the end of the test is ≤ 15% of the organic carbon added initially as the test compound.
The percent degree of biodegradation of the test item #109 BLACK during the test is detailed in the following table:

CHECK POINT (Days)	% OF BIODEGRADATION *Reference substance*	% OF BIODEGRADATION *Test item*
0	0	0
1	62	12
7	87	79
14	96	89
21	107	96
28	116	94

The trend of the degradation of both the sample and the reference, is reported in Figures N.1 and N.2.

Figure N.1: GRAPHIC OF AEROBIC BIODEGRADATION OF THE TEST ITEM DURING THE TEST

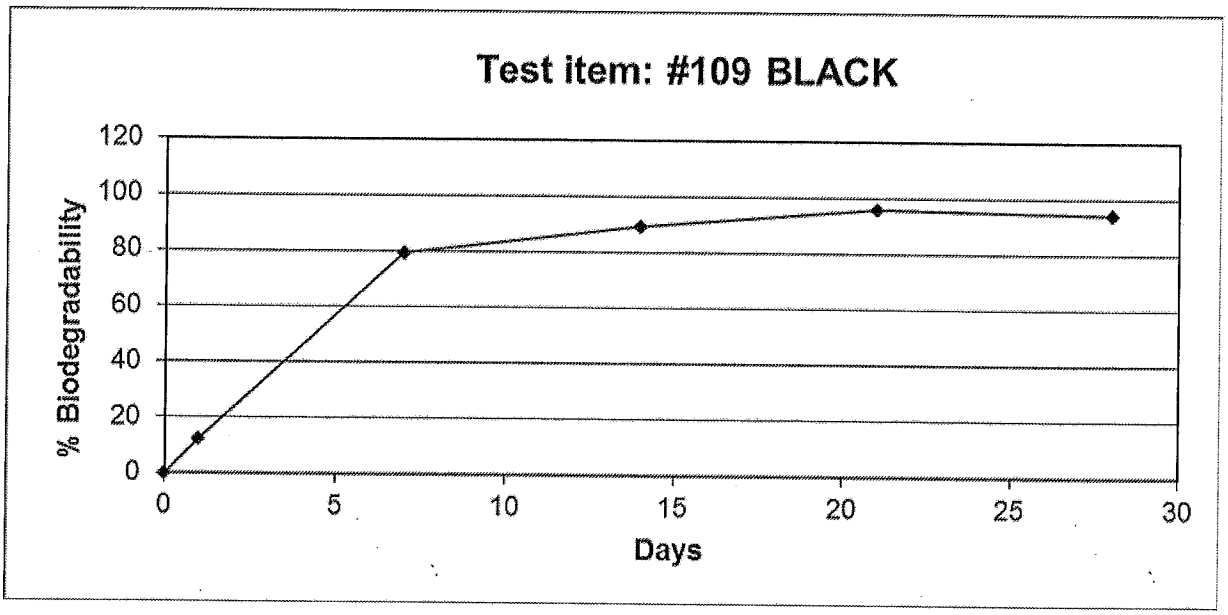
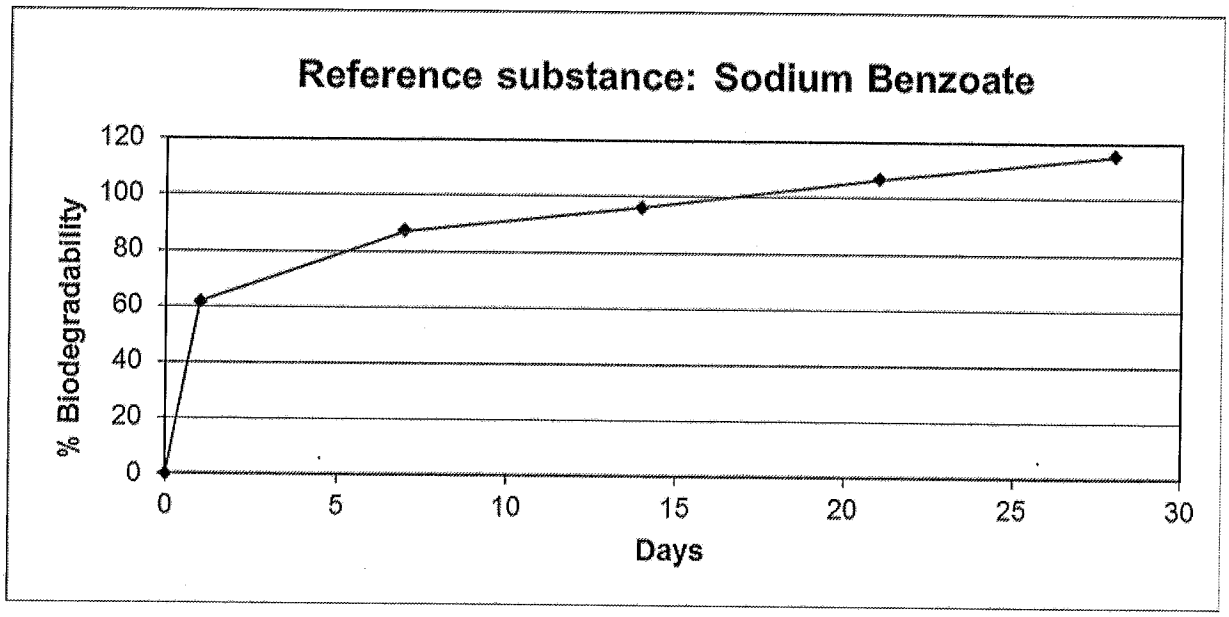


Figure N.2: GRAPHIC OF AEROBIC BIODEGRADATION OF THE REFERENCE SUBSTANCE DURING THE TEST



DEVIATIONS

No deviation has been detected during the study.

CONCLUSIONS

On the base of results obtained, interpreted in accordance to ISO 14593:1999, OECD Section 3 Part 1: 2006 the test item "#109 BLACK" should be considered **biodegradable** in aerobic condition.

ADDENDA

No addenda are enclosed.

ATTACHMENT

N.1 - Certificate of analysis N. 2017000587/LAB edited by Redox on 31/01/2017. Page: 1.

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Attachment N.1

REDOX SRL

20900 MONZA (MB) - Viale Stucchi, 62/26
Tel.: 039.2847.434 - Fax: 039.2847.349
www.labredox.com
e-mail: info@labredox.com

Analytical Chemistry & Process Safety Testing

Codice Fiscale 06709300153
Partita IVA 00834700935
R.E.A. MB 1114565
Reg. Imp. Monza Brianza 06709300153

Certificato di Analisi
Certificate of Analysis

N°Campione/ Sample N°: 2017000587/LAB

Data apertura/ Registration date: 30/01/17

Richiedente/ Requested by:
Giarei

Committente/ Company:
EUROFINS BIOLAB Srl

Campione/ Sample:
Study Code: S-2017-00040
Sample Code: ACE-2017-00013823
Name: #109 BLACK

Lotto/ Batch:
LP61022110109B

Analisi - Analysis Metodo Analitico - Analytical method	Risultati Results	Specifiche Specifications
CHN SOP - MET 019 (NOT validated method)		
Carbonio	34.15%	
Idrogeno	9.09%	
Azoto	0.66%	

Note Chiusura/ Notes:

Analisti
Analysts

Qualified Person

Data di chiusura
Date of issue
31/01/2017

Certificazione Ministero della Salute n°005/2014 (GLP)
Autorizzazione AIFA n°144/2015 (GMP)
FDA inspected (FEI 3006720740)

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