STUDY REPORT

These results concern only the samples tested in the laboratory which are defined here after.
The samples will be kept in our premises during 2 months from the date below mentioned.

masqué / redacted

June 29th 2010

EVALUATION OF AEROBIC BIODEGRADABILITY ON NATURAL LIQUID SOAP

Study promoter : masqué / redacted

Customer reference : -

EUROFINS ATS reference: **21453**

Study followed by : Eline RAYMOND

Tested product :

- Denomination : NATURAL LIQUID SOAP masqué / redacted
- Client reference : -
- ATS reference : 300740
- Brand : -

The copy of this report is only authorized by unabridged edition.
It is made of 11 pages.

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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:

Organization for Economic Co-operation and Development, ISBN 92-64-12367-9, Paris 1982

United States Food and Drug Administration, Title 21 Code of Federal Regulations Part 58, Federal Register 22 December 1978, and subsequent amendments

Italian Ministerial Decree of June 26th, 1986 "Application of Good Laboratory Practice on chemical substances and criteria for issuing the authorizations foreseen by Presidential decree n. 927/81 art. 6"

Legislative decree of January 27th, 1992, n. 120. Enforcement of Community Directives n. 88/320/CEE, n. 90/18/CEE and following decree of 5 August 1999 Enforcement of Community Directives n. 1999/11/CE and 1999/12/CE concerning inspection and verification of Good Laboratory Practice

Directive 2004/10/CE issued by European Parliament and Council dated February 11th concerning 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

Decree of the Italian Ministry of Health October 12th, 2009, Certification 85/2009 authorizing Biolab S.p.A. to perform analyses in compliance with the principles of good laboratory practices

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).

Cristina Giarei
STUDY DIRECTOR
(Dr. C.Giarei)

June 15th 2010
DATE

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CIT005 4-385

QUALITY ASSURANCE STATEMENT

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:

PHASE OF STUDY	DATE OF INSPECTION / REPORTING
Pre-experimental period	/
Experimental period	/
Post-experimental period	/
Documentation:	
- Study program	April 30th 2010
- Raw data	June 15th 2010
- Final report	June 15th 2010

Carolina Ronaghi
GLP QUALITY ASSURANCE
(Carolina Ronaghi)

June 15th 2010
DATE

SUMMARY

On the test substance, NATURAL LIQUID SOAP, the analysis of inorganic carbon for the evaluation in an aqueous medium of the aerobic biodegradability has been performed following screening method described in ISO 14593/1999 .

For this purpose the amount of Inorganic carbon developed has been reported in comparison to the blank.

The test has been done using the test substance with a concentration of about 20 mg/l of organic carbon. Samples have been kept at the temperature of 25°C for the whole period of the test (28 days).

The test product is biodegradable at 100% according to ISO 14593 and on the basis of the results obtained, interpreted according to the Regulation CE648/2004 the test substance NATURAL LIQUID SOAP can be considered biodegradable in aerobic condition.

INTRODUCTION

On behalf of Eurofins ATS. a study has been performed in order to verify the aerobic biodegradability of the test substance, in accordance with regulations of ISO 14593/1999 and CE N° 648/2004.

The study was conducted in the Biolab S.p.A. Test Facility, Vimodrone (MI) - Italy, Via Bruno Buozzi, 2.

The %C of the test substance was determined by REDOX SnC (GLP Test Facility – Authorization N. 049/2008) under the responsibility of Dr. Nebuloni as principal investigator.

EXPERIMENTATION	*START*	*END*	*RESEARCHER*
Aerobic Biodegradability of organic compounds in aqueous medium	May 05th 2010	June 10th, 2010	C. Giarei

REFERENCES

-ISO 14593: 1999. – Water quality – Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium – Method by analysis of inorganic carbon in sealed vessels (CO2 headspace test).
- EN ISO 10634:1995 – Guidance for the preparation and treatment of poorly water soluble organic compounds for the subsequent evaluation of their biodegradability in an aqueous medium.
- REGULATION (EC) No 648/2004 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 31 March 2004 on detergents.

FILING

All raw data are filed in the archives of Eurofins Biolab S.r.l for ten years after the issuing of the final report.
The control sample of the test substance will not be kept, because the Sponsor didn't provide the expiry date.
At the end of the conservation period, the Sponsor may request an extension of the conservation of all or part of the materials for a further period, or their restitution. A suitable agreement shall be drafted in this case

PROCEDURES

The procedures used in the study are documented in the Procedure Handbook of Eurofins Biolab S.r.l.

TEST PRODUCT IDENTIFICATION

The test product consist of a soap.

NAME: NATURAL LIQUID SOAP

QUALITATIVE COMPOSITION DECLARED BY THE SPONSOR: aqua, olive oil, laurel oil, potassium hydroxide, NaCl

QUANTITATIVE COMPOSITION DECLARED BY THE SPONSOR:

masqué / redacted
(secret de fabrication /
trade secret)

STABILITY: 1 year

SOLUBILITY: Soluble in water (100%)

ANALYZED SAMPLE:

The sample that was analysed, representative of the test product, consists of a green liquid contained in a plastic bottle.

Ref. N:	ALLI 01
Batch nr:	100215
Manufacture date:	March, 2010
Expiry date:	Not defined
I.D.:	10.565-S
Receiving n.:	EUITVI-8469
Receiving date:	April 22nd, 2010

Characterization of the test substance is under Sponsor responsibility

Experimental Report 2010/390 AM.A1 - Evaluation of aerobic biodegradability of organic compounds in aqueous medium

ASSAY SYSTEM

Method of sludge taking

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 inocula 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 (MI).
The plant of treatment is managed by A.L.S.I. "Alto Lambro Servizi Idrici S.p.A".

Sludge preparation

The taken sludges, when in the laboratory, have been mixed and let settle, keeping them in aerobic conditions for 6 days.
The sludge samples, before their use, have been measured the colony forming units ability and the concentration of suspended solid (about 4 mg/L) and before the beginning of the test the sludge has been centrifuged and washed.

REACTANTS

Medium

Solution A	
Diidrogenofosfato potassio anidro KH2PO4	8.50 g
Idrogenofosfato dipotassio anidro K2 HPO4	21.75 g
Idrogenofosfato disodico biidrato Na2H PO4. 2 H2O	33.40 g
Ammonium chloride NH4 Cl	0.50 g
Deionised water	q.s. to 1000 ml
Settle pH to 7.4	
Solution B	
Calcium chloride diidrato	36.40 g
Deionised water	q.s. to 1000 ml
Solution C	
Magnesium sulfate heptaidrate	22.50 g
Deionised water	q.s.to 1000 ml
Solution D	
Iron chloride (III) hexaidrate FeCl3.6H2O	0.25 g
Deionised water	q.s.to 1000 ml

To avoid precipitation some drops of concentrated HCl have been added to solution D.

MEDIUM PREPARATION: Mix 10 ml of solution A with 800 ml of deionised water and then add 1 ml of solution B, solution C and solution D and bring to 1000 ml with deionised water.

- ***Concentrated orthophosphoric acid (H3PO4) > 85% p/v***
- ***Sodium Hydroxide (Sigma-Aldrich)***
- ***Bidistilled water with contents in organic carbon <1 mg/l***

REFERENCE COMPOUND

- Sodium Benzoate (Aldrich)
Batch: 14803MH-427
Expiry date: August 2010

EQUIPMENTS AND MATERIALS

Sievers ® Carbon analyser Mod. 2244AP	GHIARONI
Orbital stirrer	GHIARONI
Centrifuge	HAEREUS
Glass bottles with hermetic seal	GHIARONI
pH meter	WTW
Filtration device	GHIARONI
Incubator	ANGELATONI SCIENTIFICA
High precision syringes	GHIARONI
Ultra turrax t25 basic	IKA WERKE

EXPERIMENTAL DESIGN

15 bottles have been prepared as follows:
- 5 bottles for the test substance
- 5 bottles for the BLANK
- 5 bottles with the reference substance (sodium benzoate)

to which the inoculum of the sludges has been added.

ASSAY EXECUTION

Preparation of the bottles

All dilutions have been done using bidistilled water.

Assay sample preparation

The elemental analysis of the test substance has been determined by REDOX SnC, and gave the following results:

ELEMENT	ANALYSIS RESULTS
Carbon	**20.41 - 20.85**
Hydrogen	8.18 - 7.99
Nitrogen	<0.1 - <0.1

The sample been used with the theoretical final concentration of 20.6 mg/l of organic carbon (TOC).

Preparation of the reference substance

Reference substance Sodium benzoate has been used with the theoretical final concentration of 20.6 mg/l of organic carbon (TOC).

Blank preparation

As blank has been used water containing culture Medium with the addition of the inoculum of the sludges.

Conditions of assay execution

The bottles prepared have been incubated at 25°C+ 2°C for about one hour for equilibration and release of gas excess.

After this period the inoculum has been added, then bottles have been closed and incubation has continued under stirring in darkness at 25°C+ 2 °C for 28 days.

The day before every determination, the reaction in one bottle of blank, one of reference substance and one of the test substance is stopped with the addition of 6 ml of sodium hydroxide (1 M).

The detection of concentration of inorganic carbon (TIC) has been measured at Time 0 and weekly.

Determination of total inorganic carbon (TIC)

The determination of inorganic carbon has been done using an automatic Sievers® analyser. It is a high sensitivity instrument based on oxidation of organic compounds that generate Carbon dioxide, by means of UV radiations and oxidizing agents. Carbon dioxide is measured using a conductimetric technique based on sensitive and selective membrane.

Samples, properly diluted with bidistilled water with low TOC contents, are analysed by means of an integrated sampling system (IOS TM) directly from vials with 40 ml volume.

For each sample under test the corresponding blank is analysed.

EXPRESSION OF RESULTS

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

Total biodegradation has been calculated using the equation:

$$Dt = \frac{(TICt - TICb)}{TOCI} * 100$$

Where:

Dt= total biodegradation calculated with reference to values of blank, expressed in percentage.

TICt = concentration of inorganic carbon, in mg, produced by biodegradation of the test substance.

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

TOCI = concentration of inorganic carbon of the test substance, 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 inorganic 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 when:
the percentage of average degradation of the reference substance is ≥ 60% after 14 days of incubation;
According to the REGULATION (EC) No 648/2004substance 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 appendix 2)
The percent degree of biodegradation of the test substance NATURAL LIQUID SOAP during the test is detailed in the following table:

ANALYSIS TIME (Days)	% OF BIODEGRADATION *Reference substance*	% OF BIODEGRADATION *Test substance*
0	0	0
1	71	41
8	100	84
15	96	91
22	124	113
28	103	90

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

DEVIATIONS

No deviation has been detected during the study.

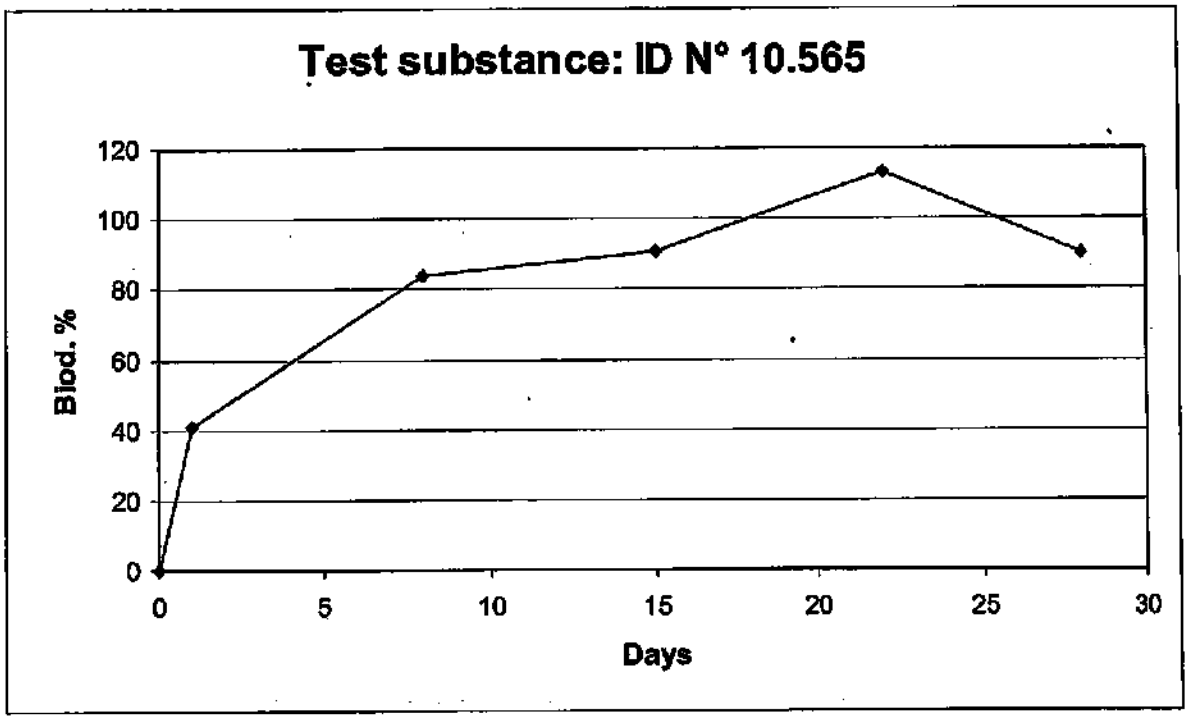
CONCLUSIONS

The test product is biodegradable at 100% according to ISO 14593 and on the basis of the results obtained, interpreted according to the Regulation CE648/2004 the test substance NATURAL LIQUID SOAP can be considered biodegradable in aerobic condition.

ADDENDA

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APPENDIX N° 1: GRAPHIC OF AEROBIC BIODEGRADATION OF THE TEST SUBSTANCE DURING THE TEST



APPENDIX N° 2: GRAPHIC OF AEROBIC BIODEGRADATION OF THE REFERENCE SUBSTANCE DURING THE TEST

