LEMI
Technopole Bordeaux-Montesquieu
33650 MARTILLAC – FRANCE

ACCREDITATION N° 1-0616 cofrac ESSAIS

Final report n° 2016-GDA-8

Evaluation of cytotoxicity
(ISO 10993-5:2009)

Medical device:
Oral Essentials DENTIFRICE (TOOTHPASTE) - BLANCHISSANT(WHITENING)
LOT : 6F15

- **Study director :** A. PEROCHE
- **Date reported :** 28 October 2016

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Pages 3 and 4 are not covered by the scope of COFRAC accreditation.

SPONSOR

HESSAM NOWZARI DDS, PHD, INC.
120 SOUTH SPALDING DRIVE
SUITE 201
BEVERLY HILLS, CALIFORNIA 90212
U.S.A.

CONTENT

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Study Director Statement and GLP

Study reference : **2016-GDA-8**

Test item: Solution of **Oral Essentials DENTIFRICE (TOOTHPASTE) - BLANCHISSANT(WHITENING) LOT : 6F15** at 5 000 µg/mL

Test : **Evaluation of cytotoxicity according to ISO 10993-5:2009 "Biological evaluation of medical devices - Tests for in vitro cytotoxicity" (LEMI SOP n° MB08/33).**

I, the undersigned, **A. PEROCHE**, study director, certify that the study was performed in the LEMI test site, and according to Good Laboratory Practice (G.L.P.) from O.E.C.D. (C(97)186 - November 26th, 1997 which Member[1] States shall recognize the assurance by another Member State that test data have been generated in accordance with GLP principles) described in ENV/MC/CHEM(98)17 (OECD Principles on Good Laboratory Practice) and described in 2004/9/EC and 2004/10/EC European directives.

Informations concerning the identity and characterization of the medical device are the responsibility of the sponsor.

I, certify that the objectives, laid down in the study plan and the associated experimental protocol, were achieved and no undesirable event occurred to affect the quality or the integrity of the study.

This report fully and accurately reflects the operating procedures used and raw data.

Date : 28 October 2016

A. PEROCHE
Study Director

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[1] List of members available on www.oecd.org

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Quality Assurance Attestation

I, the undersigned **Aurélie HORRIERE**, Quality Assurance unit of LEMI, attest that the study n° **2016-GDA-8** was submitted to the control of Quality Assurance in compliance with the principles of Good Laboratory Practice (G.L.P.).

⇒ Study plan n° **2016-GDA-8** was inspected in order to verify that it contains the necessary informations in compliance with the principles of Good Laboratory Practice.

Date of audit report inspection :	***29.09.2016***
Date of reporting to Study Director :	*29.09.2016*
Date of reporting to Test Facility Management :	*04.10.2016*

⇒ Inspections of the technical phases of the studies are carried out in LEMI. These inspections are performed in laboratory as a periodic control process of the major phases of a type of study.

Critical phase : Mycoplasma detection

Date of audit report inspection :	***26.07.2016***
Date of reporting to Study Director :	*27.07.2016*
Date of reporting to Test Facility Management :	*27.07.2016*

⇒ Test Facility inspections were carried out in LEMI. These inspections (review of procedures and applied methods) are performed to estimate the level of compliance with the principles of Good Laboratory Practice.

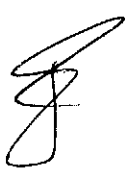
⇒ This report **2016-GDA-8** has been audited by LEMI Quality Assurance unit. It is considered to be an accurate account of the raw data generated and the application of the operating procedures in use within the laboratory :

	Draft	*Final report*
Date of audit report inspection :	***21.10.2016***	***28.10.2016***
Date of reporting to Study Director :	*21.10.2016*	*28.10.2016*
Date of reporting to Test Facility Management :	*21.10.2016*	*28.10.2016*

**(dd.mm.yyyy)*

Date : 28 October 2016

A. HORRIERE
Quality Assurance Manager



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SUMMARY

Assay : Evaluation of cytotoxicity according to ISO 10993-5 "Biological evaluation of medical devices - Tests for *in vitro* cytotoxicity" (LEMI SOP n° MB08/33).

TEST ITEM :
Solution of
Oral Essentials DENTIFRICE (TOOTHPASTE) - BLANCHISSANT(WHITENING)
LOT : 6F15
at 5 000 µg/mL of culture medium
LEMI Code : GDA051016-S2

The solution at 5 000 µg/mL of culture medium prepared from Oral Essentials DENTIFRICE (TOOTHPASTE) - BLANCHISSANT(WHITENING) LOT : 6F15 (LEMI code: GDA190916-2) according to ISO 10993-12 and ISO 10993-5, is tested to evaluate its ability to induce a cytotoxic effect in Balb/c 3T3 clone A31 cells by counting the living cells (Trypan blue exclusion test).

Controls (positive and negative) are run in parallel.

The solution at 5 000 µg/mL of culture medium prepared from Oral Essentials DENTIFRICE (TOOTHPASTE) - BLANCHISSANT(WHITENING) LOT : 6F15 (LEMI code : GDA190916-2), provided by HESSAM NOWZARI DDS, PHD, INC., is not cytotoxic.

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REPORT

1. Principle of the study

The purpose of this study is to assess the cytotoxicity of a test item (solution at 5 000 µg/mL of culture medium) in an established cell line. This study will determine the *in vitro* biological response of mammalian cells using appropriate biological parameters.

A monolayer of Balb/c 3T3 clones A31 cells (Mouse embryo fibroblasts) is exposed to the test item (solution at 5 000 µg/mL) and its dilutions for 24 hours. At the end of the incubation period, the cytotoxicity is evaluated by counting the living cells (Trypan blue exclusion test).

2. Standards

This study is performed according to :

- ISO 10993-1:2009, « Biological evaluation of Medical Devices Part. 1 – Evaluation and testing within a risk management process » ;
- ISO 10993-5:2009, « Biological evaluation of Medical Devices Part. 5 – Test for *in vitro* cytotoxicity» ;
- ISO 10993-12:2012, « Biological evaluation of Medical Devices Part. 12 – Sample preparations and reference materials ».

3. Study time-table

***Study initiation date :**	29.09.2016
***Experimental starting date :**	04.10.2016
***Experimental completion date :**	06.10.2016
***Study completion date :**	28.10.2016

**(dd.mm.yyyy)*

4. Test item identification and characterization

4.1. Medical device received

Name :	**Oral Essentials DENTIFRICE (TOOTHPASTE) - BLANCHISSANT(WHITENING) LOT : 6F15**
Container :	plastic flask
Quantity :	1
Composition :	PURIFIED WATER/AQUA, XYLITOL, HYDRATED SILICA, VEGETABLE GLYCERIN, SODIUM METHYL COCOYL TAURATE, DEAD SEA SALT, OCIMUM BASILICUM, (tulsi/holy basil) OIL, ORGANIC MENTHA PIPERITA (peppermint) OIL, MENTHA VIRIDIS (spearmint) LEAF OIL, GAULTHERIA PROCUMBENS (wintergreen) LEAF OIL, SALVIA OFFICINLAIS (sage) OIL, EUGENIA CARYOPHYLLUS (clove) FLOWER OIL, COCOS NUCIFERA (coconut) OIL, CITRUS LIMON (lemon) PEEL OIL, CARRAGEENAN (we use only food-grade carrageenan), XANTHAN GUM, ZINC CITRATE, TITANIUM DIOXIDE (CI77891)
Physical properties :	
. Aspect :	white paste
. Sterility :	non sterile
. Stability :	stable under the storage conditions
Storage conditions :	room temperature
***Expiryn date :**	-.08.2018
***Reception date :**	19.09.2016
LEMI Code :	GDA190916-2
The medical device showed no visible defect upon recept at LEMI.	

**(dd.mm.yyyy)*

4.2. Part of the medical device to be tested : NA

Name :	-
Quantity :	-
Composition :	-
Physical properties :	-
. Aspect :	-
. Sterility :	-
. Stability :	-
Storage conditions :	-
***Expiry date :**	-

**(dd.mm.yyyy)*

4.3. Solvent

Culture medium supplemented with 10 % (v/v) Fetal Calf Serum (FCS) and 1 % antibiotics (v/v).

Culture medium	**DMEM**
Supplier - Ref. - Batch :	GIBCO - 31966-021 - 1786993
Physical aspect :	liquid
Colour :	pink (pH : 7.30)
Stability :	stable under normal handling and storage conditions
FCS (Supplier – Ref. – Batch) :	GIBCO - 10270-098 - 41F0745K
Antibiotics (Supplier – Ref. – Batch) :	GIBCO - 15240-096 - 1772655
Storage conditions :	between 2° C and 8° C
Security precautions :	standard laboratory conditions

4.4. Negative control

Culture medium supplemented with 10 % (v/v) FCS and 1 % antibiotics (v/v).

Culture medium	**DMEM**
Supplier - Ref. - Batch :	GIBCO - 31966-021 - 1786993
Physical aspect :	liquid
Colour :	pink (pH : 7.30)
Stability :	stable under normal handling and storage conditions
FCS (Supplier – Ref. – Batch) :	GIBCO - 10270-098 - 41F0745K
Antibiotics (Supplier – Ref. – Batch) :	GIBCO - 15240-096 - 1772655
Storage conditions :	between 2° C and 8° C
Security precautions :	standard laboratory conditions

4.5. Positive control

Name :	**Phenol**
CAS N° :	[108-95-2]
Supplier – Ref. – Batch :	SIGMA - P5566 - BCBR0509V
Physical aspect :	powder
Colour :	white
Stability :	stable under the storage conditions
Storage conditions :	between 2 and 8°C
Security precautions :	irritant for eyes, skin and respiratory tracts
Solvent :	Culture medium (DMEM) supplemented with 10 % (v/v) FCS and 1 % antibiotics (v/v)
Concentration :	0.64 mg/mL of solvent
After solubilisation	
. Aspect	homogeneous pink solution
. Stability	extemporaneous preparation

4.6. Test item : Solution at 5 000 µg/mL of culture medium

Refer to section 6. « Preparation of the test item ».

5. Test system

Balb/c 3T3 clone A31 (ATCC CCL 163) (94th passage) tested free from mycoplasma (LEMI SOP n° MB05/02).

6. Preparation of the test item

The test item is prepared as described below :

Identification of the solution :	**GDA051016-S2**
Solvent :	Culture medium (DMEM) supplemented with 10 % (v/v) FCS and 1 % antibiotics (v/v)
Solution :	5 000 µg/mL
Dilutions :	1 500 – 500 and 150 µg/mL
Aspect :	pink red homogeneous opaque
Stability :	prepared extemporaneously
pH :	7.8 (solution at 5 000 µg/mL)
Osmolality :	347 mOsm/kg H_2O (solution at 5 000 µg/mL)

7. Assay conditions

7.1. Cell culture

Cells are seeded in multiwell plates (24 wells, 15.5 mm in diameter) at the starting density of 30 000 cells/cm² ; culture medium is DMEM supplemented with 10 % (v/v) FCS. No antibiotics are used. Cultures are incubated at 37° C in a humidified atmosphere containing 5 % (v/v) CO_2, for 24 hours.

7.2. Exposure concentrations used

Test item :	solutions	5 000 µg/mL	n* = 4
		1 500 µg/mL	n = 4
		500 µg/mL	n = 4
		150 µg/mL	n = 4
Negative control :	Complete culture medium	100 %	n = 4
Positive control :	Phenol	0.64 mg/mL	n = 4

* number of culture wells

7.3. Test

Cultures are examined with a microscope to verify that cells constitute a subconfluent monolayer and that their morphology is not altered. Culture medium is withdrawn and replaced with the solution at 5 000 µg/mL and its dilutions (1 500, 500 and 150 µg/mL), negative and positive controls supplemented with 10 % (v/v) FCS and 1 % antibiotics (v/v).

Wells are incubated at 37° C in a humidified atmosphere containing 5 % (v/v) CO_2, for a 24 hour period.

7.4. Cytotoxicity evaluation

7.4.1. Qualitative evaluation

Photos are made (x 320) showing the cell layer in contact with negative control, positive control and the solution at 5 000 µg/mL, to observe morphology and cell density of the cultures.

7.4.2. Quantitative evaluation

At the end of the incubation period, culture medium is removed. Cells are detached (2 minutes) using 250 µL trypsin (XS150916-1) (0.05 % (w/v) in Hank's balanced solution Ca^{++} and Mg^{++} free supplemented with 1 mM EDTA). Then 250 µL of a Trypan Blue (XS080816-1) solution at 0.2 % (w/v) in 0.15 M NaCl + 10 % (v/v) FCS are added (incubation for 2 minutes).

Thereafter living cells (uncolored) are counted using an haemocytometer (Malassez cell) (LEMI SOP n° MB08/23).

7.5. Expression of results

Results are expressed as number of cells per cm² (cell density).

8. Evaluation and conclusion criteria

8.1. For positive control

A statistically significant inhibition of cell growth superior to 30 % compared to the negative control must be observed.

8.2. For test item

Qualitative evaluation :

Morphology and cell density of the cell layer in contact with the solution at 5 000 µg/mL are compared to the negative control and the positive control.

Quantitative evaluation :

The inhibition of cell growth is used to evaluate the cytotoxic effect of the test item.

Statistical analysis is performed using the Student's *t* test. P values less than 0.05 are considered statistically significant. However, the biological relevance of the results shall be considered first.

A test item is considered as cytotoxic if the inhibition of the cell growth is superior to 30 %.

9. Deviation

No deviation.

10. Results

- Qualitative results are presented in photos 1 to 3, in the annex.
- Quantitative results are presented in table 1 (mean ± standard deviation), in the annex.

11. Interpretation of results

11.1.Qualitative evaluation

Photos (x 320) show quite obviously the lack of cytotoxic effect.

- Photo 1 shows the cell layer of negative control (non-cytotoxic).
- Photo 2 shows the cell layer of the positive control (cytotoxic).
- Photo 3 shows the cell layer in contact with the solution at 5 000 µg/mL : cell morphology is slightly altered and cell density is slightly lower than that of negative control.

11.2. Quantitative evaluation

- Comparison of the results obtained for :
  - the solution at 5 000 µg/mL (pH 7.8 - osmolality 347 mOsm/kg H_2O) and dilutions,
  - negative control (pH 7.7 - osmolality 345 mOsm/kg H_2O) and dilutions,

  does not show any inhibition of cell growth statistically significant superior to 30 .

According to the evaluation criteria of the cytotoxicity (see 8.2.), the solution at 5 000 µg/mL prepared from the test material is not cytotoxic.

- Positive control induces a 51 % ($P < 0.001$) inhibition on cell growth which validates the study.

Interpretation does not take into account the measurement uncertainties. These uncertainties are available and can be provided on request.

12. Conclusion

The test item (solution at 5 000 µg/mL) prepared from Oral Essentials DENTIFRICE (TOOTHPASTE) - BLANCHISSANT(WHITENING) LOT : 6F15 (LEMI code : GDA190916-2), provided by HESSAM NOWZARI DDS, PHD, INC., is not cytotoxic in the framework of ISO 10993-5 "Biological evaluation of medical devices - Tests for *in vitro* cytotoxicity".

13. Archives

Original data, correspondence, and assay report are to be kept by LEMI in a dedicated store room, for a period of ten years. At the end of this period, the assay archives will be either returned to the sponsor of the study or destroyed.

The test item is archived as a general rule 10 years, in its container of origin, whatever is its nature. However :

- the test item shall be sent back to the sponsor, at his request,
- if the sample presents a health or environmental risk, the storage conditions of the test item shall be charged to the sponsor.

Remarks :

a) The assay report above applies only to the sample of the test item submitted to the assay. Extrapolation of these results to another batch of production is the responsibility of the manufacturer.

b) This report is not to be partially duplicated without the approval of LEMI.

TECHNICAL ANNEX

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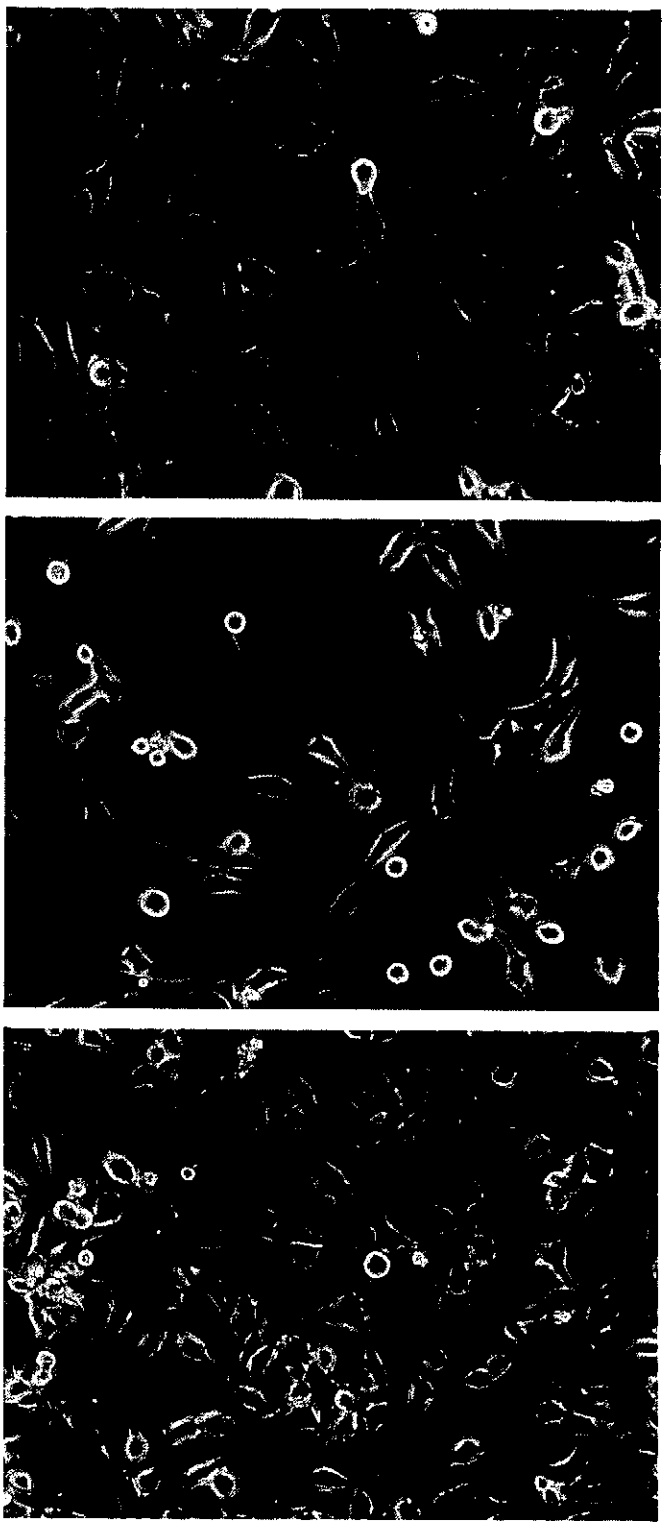
Qualitative results



1 : Negative control	1
2 : Positive control	2
3 : GDA051016-S2 – solution at 5 000 µg/mL	3

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Quantitative results

SERIE	5000 µg/mL		1500 µg/mL		500 µg/mL		150 µg/mL	
	Cells/cm²	%	Cells/cm²	%	Cells/cm²	%	Cells/cm²	%
Solution of Oral Essentials DENTIFRICE (TOOTHPASTE) - BLANCHISSANT(WHITENING) LOT : 6F15 LEMI code: GDA051016-S2	84 065 ± 6 545	79 * P < 0.01	99 220 ± 8 680	94 * NS	101 565 ± 6 930	96 * NS	101 720 ± 2 465	96 * NS
Negative control (complete culture medium)	106 095 ± 6 360	-						
Positive control (phenol 0.64 mg/mL)	52 500 ± 4 975	49 * P < 0.001						

Table n° 1

* Versus negative control
NS : not statistically significant ($P \geq 0.05$)

A test item (solution at 5 000 µg/mL) is considered as cytotoxic if the inhibition of the cell growth is superior to 30 %.

The test item (solution at 5 000 µg/mL) prepared from Oral Essentials DENTIFRICE (TOOTHPASTE) - BLANCHISSANT(WHITENING) LOT : 6F15 (LEMI code : GDA190916-2), provided by HESSAM NOWZARI DDS, PHD, INC., is not cytotoxic [2].

Date : 28 October 2016

A. PEROCHE
Study Director

[2] Accreditation of COFRAC testifies to the competence of the laboratories for only the assays covered by the accreditation.