Smart Print Bio Vitality
Batch N° PVA3 004/24

BACTERIAL REVERSE MUTATION TEST: Determination of mutagenic activity in mutated «Salmonella typhimurium his-» and «Escherichia coli»

ISO 10993-3 (October 2014), NF EN ISO 10993-3 (December 2014), ISO 10993-12 (January 2021), NF EN ISO 10993-12 (June 2021) and ISO/TR 10993-33 (March 2015) & OECD n°471 (26 June 2020)

SPONSOR	**MMTech Projetos Tecnologicos Importaçao e Exportaçao LTDA** Rua Dr Procopio Toledo Malta, 62, Morada dos Deuses São Carlos/SP ZIP 13.562-291 Brazil
SPONSOR'S REPRESENTATIVE	**Marcello DEL GUERRA**
STUDY NUMBER	**AMES-PH-24/0562**
TEST FACILITY	**LABORATOIRE ICARE - Site de Martillac - Biotox** **Technopôle Montesquieu** 14 Allée Jacques Latrille 33650 MARTILLAC FRANCE
STUDY DIRECTOR	**Alexandre PEROCHE**
STUDY COMPLETION DATE	**21 November 2024**

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STUDY DIRECTOR STATEMENT AND GLP

I, the undersigned, Alexandre PEROCHE, Study Director, certify that the study **AMES-PH-24/0562** was performed on the premises of Laboratoire ICARE - Site de Martillac.

I certify that the objectives laid down in the study plan were achieved and no undesirable event occurred to affect the quality or the integrity of the study. I consider the raw data to be valid. This report fully and accurately reflects the operating procedures used and raw data.

The entire work was planned and performed according to the principles of the Good Laboratory Practice (G.L.P.), as defined in the directive 2004/10/EC, the O.E.C.D. ruling relative to the mutual acceptance of data in the evaluation of chemical substances (C(81) 30 (final) appendices - May 12th, 1981; C (97) 186, November 26th, 1997), and in ENV/MC/CHEM (98)17.

It must be noted that the Certificate of Analysis supplied by the Sponsor (given in appendices) was generated outside the control of the Study Director and as such is not covered by this compliance statement.

Deviation to the Principles of the Good Laboratory Practice:

The purity and homogeneity of the tested batch were not supplied by the Sponsor. The Sponsor stated that the purity and homogeneity are not applicable to the test item (solid state). These deviations to GLP are under the responsibility of the Sponsor and are considered as without impact on the conclusion of the study.

The lack of verification of the concentration and stability of the test item in the vehicle during this study is a deviation to the GLP but has no impact on the reliability of the results generated for the following reasons:
- The process used to prepare the extraction of the test item was suitable according to International standard ISO 10993-12. The standard specifies that the stability must be checked only if the extracts are kept for more than 24 hours, which is not the case during this test.
- Information concerning the preparation and the visual homogeneity of the test item in vehicle are described in this report.

Date: 21 November 2024

Alexandre PEROCHE
Study Director

Quality Assurance Attestation

The study n° **AMES-PH-24/0562** was submitted to the control of Quality Assurance in compliance with the principles of Good Laboratory Practice (G.L.P.).

Quality assurance inspections are carried out according to a process of verification of critical phases during the conduct of the study.
The Study and/or the test facility and/or the process are periodically inspected by the quality assurance according to the corresponding Standard Operating Procedures.
Dates of inspections related to the different process of the study are given below:

Process	Date of inspection*	Date of reporting to study director	Date of reporting to test facility manager
Test item reception	09.08.2024	12.08.2024	05.09.2024
Test system control	29+30.07.2024	05.08.2024	19.08.2024
S9-mix preparation	04.09.2023	04.09.2023	08.09.2023
Test item preparation	02.04.2024	02.04.2024	08.04.2024
Reference item preparation	23.05.2024	07.06.2024	11.06.2024
Ames test	24+26.06.2024	04.07.2024	15.07.2024

It must be noted that these process inspection dates are not specific to this study but result from our internal audit program carried out by the Quality Assurance Unit.

This report was inspected by the Quality Assurance unit at Laboratoire ICARE – Site de Martillac.
It is considered to be an accurate account of the raw data and the application of the operating procedures in use within the laboratory.
The following inspections have been carried out in relation with this study:

Types of QA inspections	Date of inspection	Date of reporting to study director	Date of reporting to test facility manager
Study plan	18.09.2024	18.09.2024	18.09.2024
Final report	18.11.2024	18.11.2024	21.11.2024

Date: 21 November 2024

For the Quality Assurance Unit
M. CALIOT

** (dd.mm.yyyy, for all the report)*

SUMMARY

Assay: Bacterial reverse mutation test using «*Salmonella typhimurium his-*» and «*Escherichia coli*» *WP2(uvrA⁻)(pKM101)* according to ISO 10993-3 and NF EN ISO 10993-3: "Biological evaluation of medical devices Part 3- Tests for genotoxicity, carcinogenicity and reproductive toxicity" and according to OECD guideline n° 471 adapted for medical devices.

Extracts obtained from **Smart Print Bio Vitality Batch N° PVA3 004/24**, have been tested for their capacity to induce reverse mutation in four *Salmonella typhimurium* strains and one *Escherichia coli WP2(uvr A⁻)(pKM101)* strain. This study was performed in the absence and presence of metabolic activation.

Two independent assays were carried out.

For assay n°1, extracts (NaCl and Cottonseed oil) were put in contact with strains in the absence and presence of a metabolic activation system (S9-mix 5% (v/v)).

For assay n° 2, extracts (NaCl and Cottonseed oil) were put in contact with strains in the absence of metabolic activation and with pre-incubation in the presence of metabolic activation system (S9-mix 5% (v/v)).

For the two assays, negative and positive controls were carried out in parallel. Positive controls induced a significant increase in the number of revertant colonies compared to negative controls. There is no significant difference between the number of spontaneous reversions, the number of reversions obtained in the positive controls (without and with metabolic activation), and the mean of corresponding experimental "historical" values obtained in the laboratory (Table 11).

These results validate the two tests.

There is no evidence of any increase in the number of revertant colonies in the presence of the test items (original extracts: 100%/ plate) without and with metabolic activation in *Salmonella typhimurium* TA 1535, TA 1537, TA 98, TA 100 and in *Escherichia coli* WP2(uvrA⁻) (pKM 101).

Conclusion:

Extracts (NaCl 0.15M and Cottonseed oil) performed from Smart Print Bio Vitality Batch N° PVA3 004/24 (Identification code : PH-24/0562) , provided by MMTech Projetos Tecnologicos Importaçao e Exportaçao LTDA, do not induce any mutagenic change in *Salmonella typhimurium* TA 1535, TA 1537, TA 98, TA 100 and in *Escherichia coli* WP2(uvrA⁻) (pKM 101) without or with metabolic activation.

REPORT

1. PRINCIPLE OF THE STUDY

The purpose of this study was to assess the mutagenic potential of test items (pure extracts) in four *Salmonella typhimurium* strains (Ames et al)* and one mutated *Escherichia coli WP2(uvrA-) (pKM 101)* strain (Matsushima et al)**.

The assay was performed using five concentrations of the original extracts both in the absence and presence of metabolic activation by a microsomal fraction of Rat liver. After a suitable period of incubation, revertant colonies were counted and compared to the number of spontaneous revertants.

Study in absence of metabolic activation highlights direct mutagens. Study in the presence of metabolic activation highlights promutagens.

The term reverse mutation implies the mutation of *Salmonella typhimurium* strains "histidine-dependent" into " histidine-independent" *Salmonella typhimurium* strains, and the mutation "tryptophan-dependent" *Escherichia coli* strain into" tryptophan-independent" *Escherichia coli*, induced either by the substitution of base pairs, or by the addition, or deletion, of one, or more, DNA base pairs.

The determination of mutagenic activity of a test item (original extract) was its capacity to induce revertant colonies in mutated *Salmonella typhimurium* and *Escherichia coli* strains. Revertant colonies grow in absence of the amino-acid of which they were originally dependant.

2. STANDARDS

This study was conducted according to the following references:

- ISO 10993-3:2014, « Biological evaluation of Medical Devices Part. 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity ».
- NF EN ISO 10993-3:2014, «Biological evaluation of Medical Devices Part. 3 – Tests for genotoxicity, carcinogenicity and reproductive toxicity ». The European Standard EN ISO 10993-3: 2014 has the status of a French standard and fully reproduces the International Standard ISO 10993-3: 2014
- ISO 10993-12:2021, « Biological evaluation of Medical Devices Part. 12: Sample preparations and reference materials ».
- NF EN ISO 10993-12:2021, « Biological evaluation of Medical Devices Part. 12 – Sample preparations and reference materials ». The European Standard EN ISO 10993-12: 2021 has the status of a French standard and fully reproduces the International Standard ISO 10993-12: 2021
- ISO/TR 10993-33:2015, "Biological evaluation of Medical Devices Part. 33 - Guidance on tests to evaluate genotoxicity - Supplement to ISO 10993-3"
- OECD Guideline for testing of chemicals n°471 (adopted July 21st, 1997 corrected June 26th, 2020): Bacterial Reverse Mutation Test (adaptation for medical devices)

3. DEVIATIONS TO THE STUDY PLAN

No deviation.

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* Ames B.N., Mc Cann J., Yamasaki E., Methods for detecting carcinogens and mutagens with the Salmonella/Mammalian-Microsome Mutagenicity test. Mutation Research, 1975, 31, 347-364.

** Matsushima T., Takamoto Y., Shirai A., Sawamura M., Sugimura T., Reverse mutation test on 42 coded compounds with the E. coli WP 2 system. De Serres F., Ashby J. (Eds) Evaluation of short-term tests for carcinogens, Elsevier, North-Holland, 1981, 387-395.

4. STUDY TIME-TABLE*

Study initiation date:	18.09.2024
Experimental starting date:	20.09.2024
Experimental completion date:	03.10.2024
Study completion date:	21.11.2024

(dd.mm.yyyy)

	Assay 1	Assay 2
Bacteriostatic activity control :	20.09.2024 – 24.09.2024	
Sterility control :	20.09.2024 – 26.09.2024	27.09.2024 – 03.10.2024
Assay dates :	20.09.2024 – 26.09.2024	27.09.2024 – 03.10.2024

(dd.mm.yyyy)

5. TEST ITEM IDENTIFICATION AND CHARACTERIZATION

5.1. Medical Device received

Name:	**Smart Print Bio Vitality Batch N° PVA3 004/24**
Container:	See photo in appendices
Quantity:	5 bags each containing 5 units / 50x50x1.2 MM
Documents for characterization:	Test item data sheet (certificate of analysis not provided by the sponsor)
Composition:	Amorphous Silica (<5%), silanized silica (>50%), Wetting and dispersing additive (<4%), Photoinitiator (<4%), Monomeric Base (UDMA and THFMA: >40%) and Pigments (<0.07%)
Physical properties:	
. Aspect:	See photo in appendices
. Sterility:	Not sterilized
. Stability:	Stable under storage conditions
Purity and homogeneity:	NA (solid state)
Storage conditions:	Room temperature**, Keep away from light
Expiry date*:	09.04.2026
Production date*:	09.04.2024
Reception date*:	16.09.2024
Identification code:	**PH-24/0562**
	The medical device showed no visible defect upon receipt.

(dd.mm.yyyy) **(10°C to 30°C)*

Information concerning the test item is the responsibility of the Sponsor.

5.2. Test Items: Extracts obtained from the medical device received

Refer to section 7. "Extract preparation"

5.3. Negative controls - Extraction vehicles

Name	NaCl 0.15M (polar extraction vehicle)	Cottonseed oil (apolar extraction vehicle)
Supplier-Ref.-Lot	DUTSCHER - 069817B – C1273A01	SIGMA - C7767 – MKCR3880
Physical state	Liquid, limpid	Liquid, limpid
Colour	Colourless	Yellow
Storage conditions	Room temperature (RT)**	Room temperature (RT)**
Precautions of safety	No	No

Name	Dimethyl sulfoxide (DMSO)
Supplier-Ref.-Lot	SIGMA - 41639 – BCCL0999
Physical state	Liquid, limpid
Colour	Colourless
Storage conditions	Room temperature (RT)**
Precautions of safety	No

***(10°C to 30°C)*

5.4. Positive controls

Name	2-Nitrofluorene	Sodium Azide
CAS N°	[607-57-8]	[26628-22-8]
Supplier-Ref.-Lot	MERCK - N16754-5G - STBF2425V	MERCK - S2002 – STBL2894
Physical state	Powder	Powder
Colour	Beige - ecru	White
Solvent or vehicle	DMSO	NaCl 0.15M
Preparation	Extemporaneous	Extemporaneous
Stability or Expiration date*	26.01.2026	--.10.2028
Storage conditions	Room temperature (RT)**	Room temperature (RT)**
Precautions of safety	Known mutagen	Known mutagen
After solubilisation		
Aspect	Colourless solution	Colourless solution
Stability	Extemporaneous preparation	Extemporaneous preparation

Name	9-Amino acridine	2-Anthramine
CAS N°	[90-45-9]	[613-13-8]
Supplier-Ref.-Lot	MERCK - 8.18362.0010 - S8194062	MERCK - A38800 - STBJ3963
Physical state	Powder	Powder
Colour	Yellow	Yellow
Solvent or vehicle	DMSO	DMSO
Preparation	Extemporaneous	Extemporaneous
Stability or Expiration date*	30.11.2026	20.03.2026
Storage conditions	Room temperature (RT)**	Room temperature (RT)**
Precautions of safety	Known mutagen	Known mutagen
After solubilisation		
Aspect	Fluorescent iridescent blue yellow solution	Fluorescent yellow solution
Stability	Extemporaneous preparation	Extemporaneous preparation

**(dd.mm.yyyy)*
***(10°C to 30°C)*

Name	**Methyl methanesulfonate**
CAS N°	[66-27-3]
Supplier-Ref.-Lot	MERCK - 129925-5G - MKCM5645
Physical state	Liquid
Colour	Colourless
Solvent or vehicle	NaCl 0.15M
Preparation	Extemporaneous
Stability or Expiration date*	28.06.2026
Storage conditions	Room temperature (RT)**
Precautions of safety	Known mutagen
After solubilisation	
Aspect	Colourless solution
Stability	Extemporaneous preparation

**(dd.mm.yyyy)*
***(10°C to 30°C)*

6. TEST SYSTEMS AND CONTROL OF TEST SYSTEMS

6.1. Bacterial strains

Strains of Salmonella typhimurium and Escherichia coli were purchased from MOLTOX™. They were maintained in our laboratory (lyophilized discs stored at 2-8°C from TRINOVA BIOCHEM) according to internal SOP.

Genotypes are presented in the following table (see table 1):

Strain identification number	Affected gene	Complementary mutation			Mutation type
		DNA repair	Lipopoly-saccharide	R Factor	
Salmonella typhimurium TA 98	his D	ΔuvrB	rfa	pKM 101	Frame shift
Salmonella typhimurium TA 1537	his C	ΔuvrB	rfa	-	Frame shift
Salmonella typhimurium TA 100	his G	ΔuvrB	rfa	pKM 101	Base pair substitution
Salmonella typhimurium TA 1535	his G	ΔuvrB	rfa	-	Base pair substitution
Escherichia coli WP2 (uvr A⁻) (pKM101)	tryp E	ΔuvrA	-	pKM 101	Base pair substitution

Table 1: Bacterial strains

6.2. Control of strains

The genotype of bacterial strains was checked:

6.2.1. Histidine and tryptophan requirements with cultures in presence and in absence of L-histidine and L-triptophane for Salmonella typhimurium and Escherichia coli strains respectively.

6.2.2. Loss of cell wall LPS (rfa mutation) measuring crystal violet inhibition for *Salmonella typhimurium* strains.

6.2.3. Ampicillin resistance for the strains which have the pKM 101 plasmide.

6.2.4. Δuvr B mutation i.e. U.V.B sensitivity for *Salmonella typhimurium* and Δuvr A mutation i.e. U.V.A sensitivity for *Escherichia coli.*

6.2.5. Spontaneous revertant rate.

6.2.6. Sensitivity to reference mutagens (see table 2).

Strains	References items		
	- S9-mix	**+ S9-mix Without pre-incubation**	**+ S9-mix With pre-incubation**
Salmonella typhimurium TA 98	2-Nitrofluorene (2 µg/plate)	2-Anthramine (2 µg/ plate)	2-Anthramine (1 µg/ plate)
Salmonella typhimurium TA 100	Sodium azide (20 µg/ plate)	2-Anthramine (2 µg/ plate)	2-Anthramine (1 µg/ plate)
Salmonella typhimurium TA 1535	Sodium azide (5 µg/ plate)	2-Anthramine (2 µg/ plate)	2-Anthramine (1 µg/plate)
Salmonella typhimurium TA 1537	9-Aminoacridine (50 µg/ plate)	2-Anthramine (2 µg/plate)	2-Anthramine (1 µg/plate)
Escherichia coli WP2 (uvr A⁻) (pKM101)	Methyl methanesulfonate (100µg/plate)	2-Anthramine (50 µg/plate)	2-Anthramine (12.5 µg/plate)

Table 2: Reference mutagens (Positive controls)

7. EXTRACT PREPARATION

Extracts were prepared according to ISO 10993-3:2014 and ISO 10993-12:2021, NF EN ISO 10993-3:2014 et NF EN ISO 10993-12:2021.

All parts of the test item were used for the study.
No pre-treatment on the test item received, no filtration or no centrifugation and no pH adjustment on the extract obtained was performed.
The extract was used within less than 24 hours of recovery.

Extraction conditions : Extracts from **PH-24/0562**

* Extraction temperature: 37° C ± 1° C
* Extraction period: 72 h
* Gentle agitation (8 rockings/minute)
* Number of test item used: 4 (1 for NaCl 0.15M extract for assay 1, 1 for cottonseed oil extract for assay 1, 1 for NaCl 0.15M extract for assay 2 and 1 for cottonseed oil extract for assay 2)
* Area by test item: 50 cm²
* Area of test item used by extract: 50 cm²
* Extraction volume by extract: 16.7 mL
* Adsorption volume: 0 mL (volume added at the start of the extraction)
* Ratio between the surface area and the volume of extraction vehicle: 3 cm²/mL (thickness > 0.5 mm)

	NaCl extracts	
	Assay n°1	**Assay n°2**
Extraction performance	From 20.09.2024 at 09h00 to 23.09.2024 at 09h00 (72h)	From 27.09.2024 at 09h00 to 30.09.2024 at 09h00 (72h)
Start of contact extracts / test system	23.09.2024 at 10h00	30.09.2024 at 10h00
Test item **Aspect - Colour** **pH** **Osmolality**	 Limpid colourless homogeneous liquid 7.8 295 mosm/Kg H_2O	 Limpid colourless homogeneous liquid 8.3 292 mosm/Kg H_2O
Negative control **Aspect - Colour** **pH** **Osmolality**	NaCl 0.15M Limpid colourless homogeneous liquid 5.1 283 mosm/Kg H_2O	NaCl 0.15M Limpid colourless homogeneous liquid 8.1 283 mosm/Kg H_2O
	Cottonseed oil extracts	
	Assay n°1	**Assay n°2**
Extraction performance	From 20.09.2024 at 09h00 to 23.09.2024 at 09h00 (72h)	From 27.09.2024 at 09h00 to 30.09.2024 at 09h00 (72h)
Start of contact extracts / test system	23.09.2024 at 10h00	30.09.2024 at 10h00
Test item **Aspect - Colour** **pH** **Osmolality**	 Limpid / homogeneous yellow NA NA	 Limpid yellow homogeneous liquid NA NA
Negative control	Cottonseed oil	Cottonseed oil

Aspect - Colour	Limpid yellow homogeneous liquid	Limpid yellow homogeneous liquid
pH	NA	NA
Osmolality	NA	NA

Blanks: Extraction vehicle (NaCl 0.15M or Cottonseed oil), subjected to the same extraction conditions but in the absence of test material.

8. ASSAY CONDITIONS

8.1. Preparation of the metabolic activation system

8.1.1. Obtaining S9 fraction

S9 fraction, a microsome fraction prepared from Sprague Dawley rat liver homogenate, was provided by MOLTOX™ (POB Box 1189 - 157 Industrial Park Dr - Boone, NC 28607 – USA) (S9 Moltox - 11-105-5 - 4757 validated on 03.11.2023 – expiry date: 24.07.2025).

8.1.2. Preparation of S9-mix (5% v/v)

The final concentration of co-factors and salts was as follows:

S9 fraction	**5%**
$MgCl_2$-6H_2O	**8mM**
KCl	**33mM**
Glucose-6-phosphate	**5mM**
NADP	**4mM**
Phosphate buffer pH 7.4	**0.1M**

Table 3: Preparation of S9-mix

8.2. Sterility tests

Test item (original extracts) was added to 2mL of top agar maintained at 45°C and poured after homogenization on the bottom agar (20mL) onto a Petri plate (90 mm in diameter) (n = 3). Plates were incubated for 48 - 72 hours at 37°C and then examined. There should be no bacterial growth on any plate. S9-mix sterility was checked using the same protocol. Sterility of the S9-mix and the extraction vehicles were checked using the same protocol.

8.3. Bacteriostatic activity control (TA100 strain)

In a test tube 0.1m L of the bacterial suspension (1-9 x 10^3 bacteria/mL) and 0.1mL of the original extracts or these dilutions were successively added to 2mL of top agar at 45°C, containing 10% (v/v) of a solution of L-Histidine-D-Biotine (2.5mM) and 0.5 mL of sterile phosphate buffer. After homogenization, the content of the tube was poured onto a Petri plate (90 mm in diameter) containing minimal agar (20mL). 3 plates per concentration were incubated for 24-72 hours at 37°C, and the colonies counted. A negative control containing the blank alone was run in parallel.

In case bacteriostatic activity is detected, the highest concentration to be retained is that exhibiting a bacteriostatic activity of 75% or less. The precipitate, if present, should not interfere with the scoring. The following four dilutions studied are distributed according to a semi-logarithmic progression.

As only one strain (TA 100) is tested and without metabolic activation, it may be useful to test one and more concentration above the maximum concentration determined during the bacteriostatic test to observe a

cytotoxic effect for all strains and conditions tested. In this case one or more lower doses may not be tested if there is a minimum of 5 doses analysed including the additional higher doses.

8.4. Mutagenic assay

8.4.1. Assay without metabolic activation

Salmonella Typhimurium strains: for each strain, 0.1mL of the bacterial suspension containing 1-9 x10^9 bacteria/mL, 0.1mL of the original extracts and 0.5mL of sterile phosphate buffer were successively added to 2mL of overlay agar, maintained supercooled at 45°C, containing 10% (v/v) of a L-Histidine-D-Biotine solution (0.5mM).

Escherichia coli strain : in a test tube 0.1mL of the bacterial suspension containing 1-9 x 10^9 bacteria/mL and 0.1mL of the original extracts and 0.5mL of phosphate buffer were successively added to 2mL of overlay agar maintained super cooled in 45°C containing 5% (v/v) of nutrient broth n° 2 to which were added 5µl of a L-Tryptophan solution at 2 mg/mL.

After homogenization, the content of the tube was poured onto a Petri plate (90 mm in diameter) containing minimal agar (20 mL). 3 plates per concentration were incubated for 48-72 hours at 37°C and the number of revertant colonies per plate was counted.

Moreover the following controls were carried out:

- negative controls
  - absolute negative control containing no test item corresponding to the spontaneous reversion rate,
  - positive control solvent.
- blank : extraction vehicle submitted the extraction conditions without test material
- positive control (see table 2).

8.4.2. Assay with metabolic activation

Two methodologies were used:

- In assay 1, a standard plate incorporation method where the protocol is similar to that described above, except that, 500 µL of S9-mix fraction was quickly added, before pouring the mixture onto the plates;
- In assay 2, as the first assay was negative in presence of test item, the pre-incubation method was used, the test item extract with the test strain and 500 µL of S9-mix fraction were preincubated with shaking for 30 min at 37° C prior to mixing with the overlay agar and pouring onto the minimal agar plate.

This method is known to increase the detection sensitivity of a number of promutagens like alkaloids, aliphatic N-Nitroso compounds (OECD n° 471).

8.5. Presentation of data

After a 48-72 hours incubation period at 37°C, revertant colonies are counted in each plate.

Data are presented as the number of revertant colonies (mean ± standard deviation) per plate.

The following ratio is calculated:

$$R = \frac{\text{Number of revertant colonies in the presence of the test item (extract)}}{\text{Number of revertant colonies in the absence of the test item (blank)}}$$

9. EVALUATION CRITERIA

Ensure that the criteria of validity of the study are well respected namely:

- The highest concentration tested must be 100 % test sample extract or, in the event of cytotoxicity, present an "R" less than 0.5 and/or a reduction in the bacterial lawn.
- The spontaneous reversion rate of the absolute negative control shall comply with the historical values of the laboratory.
- The spontaneous reversion rate of the blank control shall comply with the historical values absolute negative control of the laboratory.
- The mean number of revertant colonies obtained for each strain and the corresponding positive control, with and/or without metabolic activation shall comply with the historical values of the laboratory.
- Negative and positive values should not show significant difference with the historical values of the laboratory.

10. CRITERIA CONCLUSION

The result of the test is considered as negative if the revertant number is below three fold the number of spontaneous reversions, for TA 1535 and TA 1537 strains, and below two fold the number of spontaneous reversions for TA 98, TA 100 and *Escherichia coli* WP2(uvrA$^-$) (pKM 101) strains without and with metabolic activation.

The result of the test is considered positive if a dependent relationship concentration is obtained in one, or several of the 5 strains, without and/or with metabolic activation, a mutagenic effect being taken into account for a given dilution of test item (extract) if the number of revertant colonies is at least two fold that of spontaneous revertant colonies for TA 98, TA 100 and *Escherichia coli* WP2(uvrA$^-$) (pKM 101), and three fold for TA 1535 and TA 1537.

All results must be confirmed in an independent experiment.

In the absence of cytotoxicity, the test can be carried out only on pure extracts.

11. RESULTS

- Results of sterility control of test items (extracts), extraction vehicles and S9-mix are presented in table 4.
- Results of bacteriostatic activity control are presented in tables 5.1 to 5.2 for the assay with the extraction vehicle NaCl 0.15M and the extraction vehicle Cottonseed oil respectively.
- Results for mutagenic activity are presented in the following tables :
    - 6.1 to 6.6 for TA 1535
    - 7.1 to 7.6 for TA 1537
    - 8.1 to 8.6 for TA 98
    - 9.1 to 9.6 for TA 100
    - 10.1 to 10.6 for *Escherichia coli*
- The means historical values of the laboratory are presented in table 11.

12. RESULT ANALYSIS

12.1. Sterility controls

12.1.1. Test items

Results presented in table 4 show the absence of any bacterial growth in the presence of the test item (extracts) and the extraction vehicles.

12.1.2. "S9-mix"

Results presented in table 4 show the absence of any bacterial growth in the presence of "S9-mix".

12.2. Bacteriostatic activity control

Results presented in tables 5.1 to 5.2 show that either original extracts or dilutions have no bacteriostatic effect.

As no bacteriostatic effect was noted, the mutagenic test was carried out only on the 100% extract.

12.3. Mutation assay results

- There is no significant difference between the number of spontaneous reversions, the number of reversions obtained for the positive controls (without and with metabolic activation), and the mean of corresponding experimental historical values obtained in the laboratory (Table 11).
- There is no evidence of any increase in the number of revertant colonies in the presence of the two test material original extracts (100% / plate) without and with metabolic activation in *Salmonella typhimurium* TA 1535, TA 1537, TA 98, TA 100 and in *Escherichia coli* WP2(uvrA$^-$) (pKM 101).
- Results are confirmed in an independent experiment.

13. CONCLUSION

In the assay conditions, in presence of extracts (NaCl 0.15M and Cottonseed oil) obtained from the test item Smart Print Bio Vitality Batch N° PVA3 004/24 (Identification code: PH-24/0562), provided by MMTech Projetos Tecnologicos Importaçao e Exportaçao LTDA, there is no evidence of any increase in the number of revertant colonies. Therefore, the test item does not induce any mutagenic change in *Salmonella typhimurium* TA 1535, TA 1537, TA 98, TA 100 strains and *Escherichia coli WP2(uvr A$^-$)(pKM101)* strain with or without metabolic activation, according to the OECD Guideline n° 471.

14. ARCHIVES

The protocol and amendments to the protocol (if any), the original data, correspondence, and final report of this short-term study are kept in a room 'salle archives" at Laboratoire ICARE - Site de Martillac for a 10-year period.
At the end of this period, the study archives will be either returned to the Sponsor of the study or destroyed.

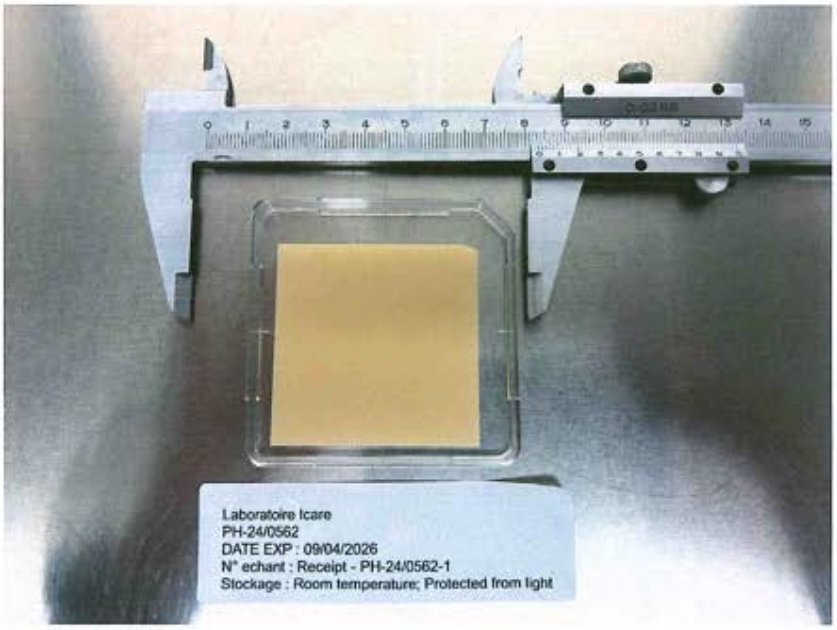
As this test is considered as a short-term study, no archive will be performed for the test item. After the end of the study, test item from its initial container will be destroyed at the sponsor's request.

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 must not be duplicated, even partially, without the approval of Laboratoire ICARE – Site de Martillac.

APPENDICES

Photo of medical device



Sterility controls

Controls	Serie	Extract concentrations	Colonies number/plate		
Control 1	Extract (NaCl) from **Smart Print Bio Vitality Batch N° PVA3 004/24**	100%	0	0	0
	Extract (Cottonseed oil) from **Smart Print Bio Vitality Batch N° PVA3 004/24**	100%	0	0	0
	Extraction vehicle : NaCl 0.15M	100%	0	0	0
	Extraction vehicle : cottonseed oil	100%	0	0	0
	S9-mix	500µL/plate	0	0	0
Control 2	Extract (NaCl) from **Smart Print Bio Vitality Batch N° PVA3 004/24**	100%	0	0	0
	Extract (Cottonseed oil) from **Smart Print Bio Vitality Batch N° PVA3 004/24**	100%	0	0	0
	Extraction vehicle : NaCl 0.15M	100%	0	0	0
	Extraction vehicle : cottonseed oil	100%	0	0	0
	S9-mix	500µL/plate	0	0	0

Table 4

Bacteriostatic activity control

		Doses (/plate)						
		Negative control	NaCl 0.15M	100%	30%	10%	3%	1%
Extract of Smart Print Bio Vitality BATCH PVA3 004/24	N1	826	811	819	801	852	891	891
	N2	812	802	841	845	846	771	773
	N3	801	819	828	789	821	854	805
	N	813 ± 13	811 ± 9	829 ± 11	812 ± 29	840 ± 16	839 ± 61	823 ± 61
	%	-	100%	102%	100%	103%	103%	101%

Table 5.1: Bacteriostatic activity control n°1 (Extract – NaCl 0.15M)

		Doses (/plate)						
		Negative control	Cottonseed oil	100%	30%	10%	3%	1%
Extract of Smart Print Bio Vitality BATCH PVA3 004/24	N1	826	857	893	785	856	836	866
	N2	812	807	764	814	841	841	791
	N3	801	801	844	863	801	774	845
	N	813 ± 13	822 ± 31	834 ± 65	821 ± 39	833 ± 28	817 ± 37	834 ± 39
	%	-	101%	103%	101%	102%	100%	103%

Table 5.2: Bacteriostatic activity control n°1 (Extract – Cottonseed oil)

N1 Number of colonies in plate 1
N2 Number of colonies in plate 2
N3 Number of colonies in plate 3
N Mean per plate
% Percent of survival compared to negative control

Mutagenic activity

TA 1535 – Controls

Serie		Dose/Plate	Plate			Mean	Standard deviation	R
			n° 1	n° 2	n° 3			
(-) S9-mix	Negative control	100 µL	9	10	12	**10,33**	**1,53**	_
	Positive control solvent	100 µL	13	12	10	**11,67**	**1,53**	_
	Positive control : Sodium azide	5 µg	937	917	843	**899,00**	**49,52**	**77,06**
(+) S9-mix 5 % without pre-incubation	Negative control	100 µL	12	15	20	**15,67**	**4,04**	_
	Positive control solvent	50 µL	14	11	13	**12,67**	**1,53**	_
	Positive control : 2-Anthramine	2 µg	129	120	136	**128,33**	**8,02**	**10,13**

Table 6.1: Assay n°1 (Controls)

Plate, Mean and Standard Deviation unity : number of revertant colonies/plate

R = Number of revertant colonies in the presence of the test item /Number of revertant colonies in the absence of the test item

Serie		Dose/Plate	Plate			Mean	Standard deviation	R
			n° 1	n° 2	n° 3			
(-) S9-mix	Negative control	100 µL	10	14	13	**12,33**	**2,08**	_
	Positive control solvent	100 µL	10	12	13	**11,67**	**1,53**	_
	Positive control : Sodium azide	5 µg	358	382	368	**369,33**	**12,06**	**31,66**
(+) S9-mix 5 % with pre-incubation	Negative control	100 µL	15	13	6	**11,33**	**4,73**	_
	Positive control solvent	50 µL	11	13	10	**11,33**	**1,53**	_
	Positive control : 2-Anthramine	1 µg	109	161	108	**126,00**	**30,32**	**11,12**

Table 6.2: Assay n°2 (Controls)

TA 1535 – Extracts NaCl 0.15M

Serie		Dose/Plate	Plate			Mean	Standard deviation	R
			n° 1	n° 2	n° 3			
(-) S9-mix	Vehicle :	100 µL	15	15	8	**12,67**	**4,04**	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	8	9	16	**11,00**	**4,36**	**0,87**
(+) S9-mix 5 % without pre-incubation	Vehicle :	100 µL	9	13	11	**11,00**	**2,00**	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	11	11	9	**10,33**	**1,15**	**0,94**

Table 6.3: Assay n° 1 (Extract – NaCl 0.15M)

Serie		Dose/Plate	Plate			Mean	Standard deviation	R
			n° 1	n° 2	n° 3			
(-) S9-mix	Vehicle :	100 µL	18	5	12	**11,67**	**6,51**	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	10	11	9	**10,00**	**1,00**	**0,86**
(+) S9-mix 5 % with pre-incubation	Vehicle :	100 µL	14	3	5	**7,33**	**5,86**	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	11	13	15	**13,00**	**2,00**	**1,77**

Table 6.4: Assay n° 2 (Extract – NaCl 0.15M)

Background lawn observation code:
(1) Normal.

TA 1535 – Extracts Cottonseed oil

Serie		Dose/Plate	Plate			Mean	Standard deviation	R
			n° 1	n° 2	n° 3			
(-) S9-mix	Vehicle :	100 µL	12	10	9	10,33	1,53	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	10	9	10	9,67	0,58	0,94
(+) S9-mix 5 % without pre-incubation	Vehicle :	100 µL	10	11	9	10,00	1,00	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	15	8	11	11,33	3,51	1,13

Table 6.5: Assay n° 1 (Extract – Cottonseed oil)

Serie		Dose/Plate	Plate			Mean	Standard deviation	R
			n° 1	n° 2	n° 3			
(-) S9-mix	Vehicle :	100 µL	6	11	17	11,33	5,51	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	9	10	7	8,67	1,53	0,76
(+) S9-mix 5 % with pre-incubation	Vehicle :	100 µL	12	16	4	10,67	6,11	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	13	14	7	11,33	3,79	1,06

Table 6.6: Assay n° 2 (Extract – Cottonseed oil)

Background lawn observation code:
(1) Normal.

TA 1537 – Controls

Serie		Dose/Plate	Plate			Mean	Standard deviation	R
			n° 1	n° 2	n° 3			
(-) S9-mix	Negative control	100 µL	6	8	10	**8,00**	**2,00**	_
	Positive control solvent	50 µL	7	7	12	**8,67**	**2,89**	_
	Positive control : 9-Aminoacridine	50 µg	1037	1334	1512	**1294,33**	**239,97**	**149,35**
(+) S9-mix 5 % without pre-incubation	Negative control	100 µL	13	9	10	**10,67**	**2,08**	_
	Positive control solvent	50 µL	14	10	11	**11,67**	**2,08**	_
	Positive control : 2-Anthramine	2 µg	94	103	97	**98,00**	**4,58**	**8,40**

Table 7.1: Assay n°1 (Controls)

Serie		Dose/Plate	Plate			Mean	Standard deviation	R
			n° 1	n° 2	n° 3			
(-) S9-mix	Negative control	100 µL	4	7	9	**6,67**	**2,52**	_
	Positive control solvent	50 µL	7	10	8	**8,33**	**1,53**	_
	Positive control : 9-Aminoacridine	50 µg	907	855	806	**856,00**	**50,51**	**102,72**
(+) S9-mix 5 % with pre-incubation	Negative control	100 µL	4	12	13	**9,67**	**4,93**	_
	Positive control solvent	50 µL	13	8	11	**10,67**	**2,52**	_
	Positive control : 2-Anthramine	1 µg	77	68	71	**72,00**	**4,58**	**6,75**

Table 7.2: Assay n°2 (Controls)

TA 1537 – Extracts NaCl 0.15M

Serie		Dose/Plate	Plate			Mean	Standard deviation	R
			n° 1	n° 2	n° 3			
(-) S9-mix	Vehicle :	100 µL	10	11	8	9,67	1,53	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	7	9	8	8,00	1,00	0,83
(+) S9-mix 5 % without pre-incubation	Vehicle :	100 µL	10	12	10	10,67	1,15	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	15	12	10	12,33	2,52	1,16

Table 7.3: Assay n° 1 (Extract – NaCl 0.15M)

Serie		Dose/Plate	Plate			Mean	Standard deviation	R
			n° 1	n° 2	n° 3			
(-) S9-mix	Vehicle :	100 µL	12	4	7	7,67	4,04	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	5	4	7	5,33	1,53	0,70
(+) S9-mix 5 % with pre-incubation	Vehicle :	100 µL	9	14	6	9,67	4,04	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	5	9	6	6,67	2,08	0,69

Table 7.4: Assay n° 2 (Extract – NaCl 0.15M)

Background lawn observation code:
(1) Normal.

TA 1537 – Extracts Cottonseed oil

Serie		Dose/Plate	Plate			Mean	Standard deviation	R
			n° 1	n° 2	n° 3			
(-) S9-mix	Vehicle :	100 µL	7	9	7	7,67	1,15	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	6	9	7	7,33	1,53	0,96
(+) S9-mix 5 % without pre-incubation	Vehicle :	100 µL	11	7	12	10,00	2,65	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	10	14	11	11,67	2,08	1,17

Table 7.5: Assay n° 1 (Extract – Cottonseed oil)

Serie		Dose/Plate	Plate			Mean	Standard deviation	R
			n° 1	n° 2	n° 3			
(-) S9-mix	Vehicle :	100 µL	4	7	9	6,67	2,52	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	7	9	5	7,00	2,00	1,05
(+) S9-mix 5 % with pre-incubation	Vehicle :	100 µL	5	13	13	10,33	4,62	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	9	3	11	7,67	4,16	0,74

Table 7.6: Assay n° 2 (Extract – Cottonseed oil)

Background lawn observation code:
(1) Normal.

TA 98 – Controls

Serie		Dose/Plate	Plate			Mean	Standard deviation	R
			n° 1	n° 2	n° 3			
(-) S9-mix	Negative control	100 µL	17	19	16	**17,33**	**1,53**	_
	Positive control solvent	50 µL	14	17	17	**16,00**	**1,73**	_
	Positive control : 2-Nitrofluorene	2 µg	341	245	318	**301,33**	**50,12**	**18,83**
(+) S9-mix 5 % without pre-incubation	Negative control	100 µL	34	30	17	**27,00**	**8,89**	_
	Positive control solvent	50 µL	31	26	23	**26,67**	**4,04**	_
	Positive control : 2-Anthramine	2 µg	1317	1374	1394	**1361,67**	**39,95**	**51,06**

Table 8.1: Assay n°1 (Controls)

Serie		Dose/Plate	Plate			Mean	Standard deviation	R
			n° 1	n° 2	n° 3			
(-) S9-mix	Negative control	100 µL	20	20	14	**18,00**	**3,46**	_
	Positive control solvent	50 µL	24	22	20	**22,00**	**2,00**	_
	Positive control : 2-Nitrofluorene	2 µg	463	411	327	**400,33**	**68,62**	**18,20**
(+) S9-mix 5 % with pre-incubation	Negative control	100 µL	37	35	30	**34,00**	**3,61**	_
	Positive control solvent	50 µL	22	34	27	**27,67**	**6,03**	_
	Positive control : 2-Anthramine	1 µg	1051	1143	1224	**1139,33**	**86,56**	**41,18**

Table 8.2: Assay n°2 (Controls)

TA 98 – Extracts NaCl 0.15M

Serie		Dose/Plate	Plate			Mean	Standard deviation	R
			n° 1	n° 2	n° 3			
(-) S9-mix	Vehicle :	100 µL	13	15	18	**15,33**	**2,52**	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	17	19	24	**20,00**	**3,61**	**1,30**
(+) S9-mix 5 % without pre-incubation	Vehicle :	100 µL	33	17	26	**25,33**	**8,02**	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	26	28	33	**29,00**	**3,61**	**1,14**

Table 8.3: Assay n° 1 (Extract – NaCl 0.15M)

Serie		Dose/Plate	Plate			Mean	Standard deviation	R
			n° 1	n° 2	n° 3			
(-) S9-mix	Vehicle :	100 µL	17	15	13	**15,00**	**2,00**	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	17	20	15	**17,33**	**2,52**	**1,16**
(+) S9-mix 5 % with pre-incubation	Vehicle :	100 µL	34	22	18	**24,67**	**8,33**	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	23	30	24	**25,67**	**3,79**	**1,04**

Table 8.4: Assay n° 2 (Extract – NaCl 0.15M)

Background lawn observation code:
(1) Normal.

TA 98 – Extracts Cottonseed oil

Serie		Dose/Plate	Plate			Mean	Standard deviation	R
			n° 1	n° 2	n° 3			
(-) S9-mix	Vehicle :	100 µL	11	12	8	**10,33**	**2,08**	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	14	13	10	**12,33**	**2,08**	**1,19**
(+) S9-mix 5 % without pre-incubation	Vehicle :	100 µL	31	20	24	**25,00**	**5,57**	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	24	23	29	**25,33**	**3,21**	**1,01**

Table 8.5: Assay n° 1 (Extract – Cottonseed oil)

Serie		Dose/Plate	Plate			Mean	Standard deviation	R
			n° 1	n° 2	n° 3			
(-) S9-mix	Vehicle :	100 µL	13	10	7	**10,00**	**3,00**	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	13	9	8	**10,00**	**2,65**	**1,00**
(+) S9-mix 5 % with pre-incubation	Vehicle :	100 µL	16	23	19	**19,33**	**3,51**	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	22	31	29	**27,33**	**4,73**	**1,41**

Table 8.6: Assay n° 2 (Extract – Cottonseed oil)

Background lawn observation code:
(1) Normal.

TA 100 – Controls

Serie		Dose/Plate	Plate			Mean	Standard deviation	R
			n° 1	n° 2	n° 3			
(-) S9-mix	Negative control	100 µL	76	69	71	72,00	3,61	_
	Positive control solvent	100 µL	66	61	61	62,67	2,89	_
	Positive control : Sodium Azide	20 µg	1270	1225	1089	1194,67	94,24	19,06
(+) S9-mix 5 % without pre-incubation	Negative control	100 µL	67	90	61	72,67	15,31	_
	Positive control solvent	50 µL	63	66	57	62,00	4,58	_
	Positive control : 2-Anthramine	2 µg	795	786	876	819,00	49,57	13,21

Table 9.1: Assay n°1 (Controls)

Serie		Dose/Plate	Plate			Mean	Standard deviation	R
			n° 1	n° 2	n° 3			
(-) S9-mix	Negative control	100 µL	83	67	72	74,00	8,19	_
	Positive control solvent	100 µL	69	49	72	63,33	12,50	_
	Positive control : Sodium Azide	20 µg	1157	1039	1046	1080,67	66,20	17,06
(+) S9-mix 5 % with pre-incubation	Negative control	100 µL	75	67	83	75,00	8,00	_
	Positive control solvent	50 µL	81	65	56	67,33	12,66	_
	Positive control : 2-Anthramine	1 µg	722	616	544	627,33	89,54	9,32

Table 9.2: Assay n°2 (Controls)

TA 100 – Extracts NaCl 0.15M

Serie		Dose/Plate	Plate			Mean	Standard deviation	R
			n° 1	n° 2	n° 3			
(-) S9-mix	Vehicle :	100 µL	67	73	80	73,33	6,51	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	86	70	58	71,33	14,05	0,97
(+) S9-mix 5 % without pre-incubation	Vehicle :	100 µL	63	75	80	72,67	8,74	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	59	62	65	62,00	3,00	0,85

Table 9.3: Assay n° 1 (Extract – NaCl 0.15M)

Serie		Dose/Plate	Plate			Mean	Standard deviation	R
			n° 1	n° 2	n° 3			
(-) S9-mix	Vehicle :	100 µL	66	79	70	71,67	6,66	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	77	68	70	71,67	4,73	1,00
(+) S9-mix 5 % with pre-incubation	Vehicle :	100 µL	56	82	70	69,33	13,01	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	75	80	79	78,00	2,65	1,13

Table 9.4: Assay n° 2 (Extract – NaCl 0.15M)

Background lawn observation code:
(1) Normal.

TA 100 – Extracts Cottonseed oil

Serie		Dose/Plate	Plate			Mean	Standard deviation	R
			n° 1	n° 2	n° 3			
(-) S9-mix	Vehicle :	100 µL	63	58	61	60,67	2,52	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	71	68	76	71,67	4,04	1,18
(+) S9-mix 5 % without pre-incubation	Vehicle :	100 µL	65	71	75	70,33	5,03	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	75	68	78	73,67	5,13	1,05

Table 9.5: Assay n° 1 (Extract – Cottonseed oil)

Serie		Dose/Plate	Plate			Mean	Standard deviation	R
			n° 1	n° 2	n° 3			
(-) S9-mix	Vehicle :	100 µL	79	68	71	72,67	5,69	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	51	65	62	59,33	7,37	0,82
(+) S9-mix 5 % with pre-incubation	Vehicle :	100 µL	69	71	58	66,00	7,00	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	77	75	61	71,00	8,72	1,08

Table 9.6: Assay n° 2 (Extract – Cottonseed oil)

Background lawn observation code:
(1) Normal.

E. COLI – Controls

Serie		Dose/Plate	Plate			Mean	Standard deviation	R
			n° 1	n° 2	n° 3			
(-) S9-mix	Negative control	100 µL	45	42	51	**46,00**	**4,58**	_
	Positive control solvent	100 µL	68	60	50	**59,33**	**9,02**	_
	Positive control : MMS	1 µg	365	343	335	**347,67**	**15,53**	**5,86**
(+) S9-mix 5 % without pre-incubation	Negative control	100 µL	107	116	101	**108,00**	**7,55**	_
	Positive control solvent	50 µL	71	101	101	**91,00**	**17,32**	_
	Positive control : 2-Anthramine	50 µg	1138	1139	1200	**1159,00**	**35,51**	**12,74**

Table 10.1: Assay n°1 (Controls)

Serie		Dose/Plate	Plate			Mean	Standard deviation	R
			n° 1	n° 2	n° 3			
(-) S9-mix	Negative control	100 µL	49	47	46	**47,33**	**1,53**	_
	Positive control solvent	100 µL	44	47	56	**49,00**	**6,24**	_
	Positive control : MMS	1 µg	484	457	408	**449,67**	**38,53**	**9,18**
(+) S9-mix 5 % with pre-incubation	Negative control	100 µL	75	79	68	**74,00**	**5,57**	_
	Positive control solvent	50 µL	105	81	107	**97,67**	**14,47**	_
	Positive control : 2-Anthramine	12.5 µg	720	950	882	**850,67**	**118,16**	**8,71**

Table 10.2: Assay n°2 (Controls)

E. COLI – Extracts NaCl 0.15M

Serie		Dose/Plate	Plate			Mean	Standard deviation	R
			n° 1	n° 2	n° 3			
(-) S9-mix	Vehicle :	100 µL	51	65	52	**56,00**	**7,81**	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	41	64	59	**54,67**	**12,10**	**0,98**
(+) S9-mix 5 % without pre-incubation	Vehicle :	100 µL	77	86	108	**90,33**	**15,95**	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	79	94	65	**79,33**	**14,50**	**0,88**

Table 10.3: Assay n° 1 (Extract – NaCl 0.15M)

Serie		Dose/Plate	Plate			Mean	Standard deviation	R
			n° 1	n° 2	n° 3			
(-) S9-mix	Vehicle :	100 µL	53	51	63	**55,67**	**6,43**	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	44	49	55	**49,33**	**5,51**	**0,89**
(+) S9-mix 5 % with pre-incubation	Vehicle :	100 µL	91	107	105	**101,00**	**8,72**	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	97	72	83	**84,00**	**12,53**	**0,83**

Table 10.4: Assay n° 2 (Extract – NaCl 0.15M)

Background lawn observation code:
(1) Normal.

E. COLI – Extracts Cottonseed oil

Serie		Dose/Plate	Plate			Mean	Standard deviation	R
			n° 1	n° 2	n° 3			
(-) S9-mix	Vehicle :	100 µL	52	63	50	55,00	7,00	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	51	60	62	57,67	5,86	1,05
(+) S9-mix 5 % without pre-incubation	Vehicle :	100 µL	76	81	85	80,67	4,51	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	75	79	80	78,00	2,65	0,97

Table 10.5: Assay n° 1 (Extract – Cottonseed oil)

Serie		Dose/Plate	Plate			Mean	Standard deviation	R
			n° 1	n° 2	n° 3			
(-) S9-mix	Vehicle :	100 µL	52	61	53	55,33	4,93	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	47	52	50	49,67	2,52	0,90
(+) S9-mix 5 % with pre-incubation	Vehicle :	100 µL	77	79	81	79,00	2,00	_
	Extract of Smart Print Bio Vitality BATCH PVA3 004/24	100% (1)	101	75	88	88,00	13,00	1,11

Table 10.6: Assay n° 2 (Extract – Cottonseed oil)

Background lawn observation code:
(1) Normal.

Historical control values

Historical control values - Ames test

2014 to 2023		Without metabolic activation					With metabolic activation (without pre-incubation)					With metabolic activation (with pre-incubation)				
		n	Mean		min	/ max	*n*	Mean		min	/ max	*n*	Mean		min	/ max
Salmonella typhimurium TA 1535	Spontaneous reversion	*1146*	**11.1**	± 3.8	2.0	/ 23.0	*594*	**11.7**	± 3.9	2.0	/ 23.0	*573*	**12.1**	± 4.1	4.0	/ 25.0
	Sodium Azide	*1146*	**810.4**	± 192.6	190.0	/ 1487.0	*594*	**130.1**	± 46.4	28.0	/ 269.0	*570*	**89.2**	± 47.2	25.0	/ 471.0
Salmonella typhimurium TA 1537	Spontaneous reversion	*1146*	**6.3**	± 2.9	1.0	/ 20.0	*600*	**8.4**	± 3.9	1.0	/ 24.0	*573*	**8.6**	± 3.9	1.0	/ 21.0
	9-Aminoacridine	*1146*	**1032.5**	± 391.0	224.0	/ 1978.0	*600*	**61.9**	± 28.7	21.0	/ 181.0	*570*	**50.3**	± 25.8	19.0	/ 184.0
Salmonella typhimurium TA 98	Spontaneous reversion	*1146*	**16.4**	± 4.6	6.0	/ 29.0	*597*	**24.3**	± 6.2	10.0	/ 38.0	*570*	**23.8**	± 6.2	10.0	/ 37.0
	2-Nitrofluorene	*1146*	**422.9**	± 161.1	167.0	/ 1532.0	*597*	**644.5**	± 255.7	220.0	/ 1478.0	*573*	**443.3**	± 199.4	153.0	/ 1368.0
Salmonella typhimurium TA 100	Spontaneous reversion	*1146*	**66.3**	± 12.5	40.0	/ 121.0	*594*	**82.2**	± 14.9	51.0	/ 150.0	*570*	**81.9**	± 14.9	49.0	/ 156.0
	Sodium Azide	*1146*	**1221.3**	± 243.6	473.0	/ 1955.0	*594*	**854.9**	± 359.9	256.0	/ 2163.0	*570*	**572.3**	± 244.9	274.0	/ 1720.0
Escherichia coli WP2 (pKM 101) (uvr A-)	Spontaneous reversion	*1098*	**89.2**	± 27.0	41.0	/ 211.0	*573*	**132.9**	± 36.1	60.0	/ 263.0	*558*	**133.2**	± 33.7	61.0	/ 251.0
	MMS	*267*	**564.0**	± 174.8	218.0	/ 1518.0	*159*	**963.1**	± 264.0	435.0	/ 1725.0	*144*	**984.6**	± 252.4	403.0	/ 1562.0

Table 11: Historical control values (2014 to 2023)

n = number of plates.

TRINOVABIOCHEM

MOLTOX®
Molecular Toxicology, Inc.

POST MITOCHONDRIAL SUPERNATANT (S9)
QUALITY CONTROL & PRODUCTION CERTIFICATE

Animal Information
SPECIES: Rat
STRAIN: Sprague Dawley
SEX: Male
AGE: 5 – 6 weeks
WEIGHT: 175 – 199 g
TISSUE: Liver

Part Number Information
LOT NO.: 4757
PART NO.: 11-105
VOLUME: 5 mL
BUFFER: 0.15 M KCl
STORAGE: At or below -70°C

PREP: July 24, 2023
EXPIRY: July 24, 2025
INDUCING AGENT:
Phenobarbital-5,6
Benzoflavone

REFERENCE: Matsushima, et al., *In Vitro Metabolic Activation in Mutagenesis Testing* (F.J. de Serres, ed.), Elsevier, 1976, p 85

For Research Purposes Only

BIOCHEMISTRY:
- PROTEIN: 38.6 mg/ml — Assayed according to the method of Lowry et al., *JBC* 193:265, 1951 using bovine serum albumin as the standard.

- ALKOXYRESORUFIN-O-DEALKYLASE ACTIVITIES

Activity	P450	Fold-Induction
BROD	2B1, 2B2	87
EROD	1A1, 1A2	82.2
MROD	1A1, 1A2	16.6
PROD	2B1, 2B2	45.6

Assays for ethoxyresorufin-O-deethylase (EROD), pentoxy-, benzyl- and methoxyresorufin-O-dealkylases (PROD, BROD, & PROD, MROD) were conducted using a modification of the methods of Burke, et al., *Biochem Pharm* 34:3337, 1985. Fold-inductions were calculated as the ratio of the sample vs. uninduced specific activities (SA's). Control SA's (pmoles/min/ mg protein) were 103.4, 92.8, 47.1, & 33.6 for BROD, EROD, MROD and PROD, respectively.

BIOASSAY:
- TEST FOR THE PRESENCE OF ADVENTITIOUS AGENTS

Samples of S-9 were assayed for the presence of contaminating microorganisms by plating 1.0 ml volumes on Nutrient Agar and Minimal Glucose (Vogel-Bonner E, supplemented with 0.05 mM L-histidine and D-biotin) media. Duplicate plates were read after 40 - 48 h incubation at 35 ± 2°C. The tested samples met acceptance criteria.

- PROMUTAGEN ACTIVATION

No. His+ Revertants	
TA98	TA1535
191.2	1484

The ability of the sample to activate ethidium bromide (EtBr) and cyclophosphamide (CPA) to intermediates mutagenic to TA98 and TA1535, respectively, was determined according to Lesca, et al., *Mutation Res* 129: 299, 1984. Data were expressed as revertants per µg EtBr or per mg CPA.

Dilutions of the sample S9, ranging from 0.2 – 10% in S9 mix, were tested for their ability to activate benzo(α)pyrene (BP) and 2-aminoanthracene (2-AA) to metabolites mutagenic to TA100. Assays were conducted as described by Maron & Ames, (*Mutat Res* 113: 173, 1983.)

	µl S9 per plate/number Avg* revertants per plate					
Promutagen	0	1	5	10	20	50
BP (5 µg)	139	143	221	275	397	571
AA (2.5 µg)	83	142	501	1051	1918	1537

Approved: 07/28/2023

MOLECULAR TOXICOLOGY, INC. www.moltox.com (828) 264-9099

TRINOVA BIOCHEM GmbH
Rathenau Str. 2
35394 Giessen
GERMANY

fon +49 (0) 641 94390-0
fax +49 (0) 641 94390-22
info@trinova.de
www.trinova.de

ansm
Agence nationale de sécurité du médicament et des produits de santé

RÉPUBLIQUE FRANÇAISE

Direction de l'inspection

EVALUATION DE LA CONFORMITE AUX BONNES PRATIQUES DE LABORATOIRE

selon la directive 2004/9/CE (ESSAIS DE SECURITE)
STATEMENT OF COMPLIANCE WITH GOOD LABORATORY PRACTICES
according to Directive 2004/9/CE (SAFETY TESTS)

Nom et adresse de l'installation d'essai :
Name and location of the test facility

Laboratoire ICARE -Site de Martillac
Technopôle Montesquieu
14 Allée Jacques Latrille
33650 MARTILLAC

Objet de l'inspection : *Purpose of the inspection*
état des lieux : ☒ *Test facility inspection*
vérification d'étude(s) : ☒ *Study audit*

Date(s) d'inspection : *Date(s) of the inspection*
10 au 13 janvier 2023

Degré de conformité aux B.P.L. * : *Status*
Conforme

Degré de conformité aux BPL valant pour les études achevées entre le 24 septembre 2021 et le 13 janvier 2023
Level of compliance with GLP for studies performed between

Catégorie(s) d'éléments d'essai : *Types of Test items :*
Dispositifs médicaux
Medical devices

Domaine d'activité : *Areas of expertise*
Cytotoxicité, sensibilisation cutanée, irritations et corrosions cutanées, oculaires et des muqueuses, réactivité intradermique, hémocompatibilité, pyrogénicité, toxicité systémique aiguë, subchronique et subaiguë et toxicité chronique, essais d'implantation et génotoxicité..

Catégorie OCDE (appendice à l'annexe III de C(89)87(Final)/révisée dans C(95)8(Final))
OECD category

1	2	3	4	5	6	7	8	9

Commentaires éventuels : néant.
Observations (if applicable)

Fait à Saint-Denis (France), le : 24 février 2023
Date of the statement

Frederique MARCHAL
Signature numérique de Frederique MARCHAL
Date : 2023.02.24 15:57:15 +01'00'

Cheffe du pôle Inspection des essais et des vigilances

* Conforme: conformité aux B.P.L. (*in conformity with GLP*);
Non-Conforme :absence de conformité aux B.P.L. (*not in conformity with GLP*).

Code : DOC_524_v01

143/147 boulevard Anatole France - F-93285 Saint-Denis Cedex - Tél. +33 (0)1 55 87 30 00 - www.ansm.sante.fr