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Final Study Report

CONFIDENTIAL

***In Vivo* Erythrocytes-BASED *Pig-A* GENE MUTATION ASSAY**
(Performed in Mouse Somatic Cells - Two sampling times)
Combined to the
***In Vivo* MAMMALIAN ALKALINE COMET ASSAY**
(Performed in Mouse Circulating Blood Cells - One sampling time)

(Five treatments followed by 3 co-treatments)

Study Number
FSR-IPL 160901

Study Completion
17 February 2017

Test Item
ADN Telomeractives®

Study Director
Dr. Sophie SIMAR

Sponsor
HBN

TEST FACILITY
INSTITUT PASTEUR DE LILLE
Genetic Toxicology Laboratory
1, rue du Professeur Calmette - BP. 245
59019 LILLE CEDEX

SPONSOR
HBN
1 rue des pénitents blancs
31010 TOULOUSE

SPONSOR REPRESENTATIVE
Mr. Gérald GOUDET

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT AND REPORT AUTHENTICATION

* * *

The work described in this report was performed according to the agreed study plan and with the Standard Operating Procedures of the test facility, unless otherwise stated, and was conducted in accordance with:

- OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17;
- GLP departmental order 14/3/2000 (Official Journal of 23rd March 2000);
- EC Commission Directive 2004/10/EC of 11th February 2004 (Official Journal No. L050);
- Application of the OECD Principles of GLP to Computerised Systems, No. 10 Consensus Document of the Working Group on Good Laboratory Practice, OECD/GD(95)115.

I consider the data generated and reported to be valid and I declare that this report is a true and accurate record of the results obtained.

As described in the Study Plan, the sponsor certifies that the test item to be tested provided by **HBN** is identical to the test item described in the Final Study Plan.

Note: No Analytical Certificate of the test item was provided.

No data about Composition (or concentration) or Stability in storage conditions was provided. This constitutes a deviation to the Good Laboratory Practices (OECD, 1997: § 6.2, Characterisation).

No control of concentration in dosing formulation was performed. This also constitutes a deviation to the recommendations of the Good Laboratory Practices (OECD, 1997: §6.2, Characterisation).

Nevertheless, taking into account the nature of the test item (*i.e.* plant extract), this deviation was considered only as a minor deviation.

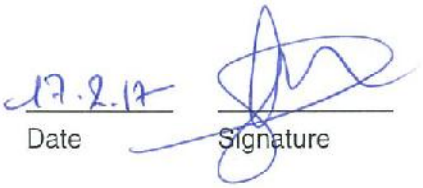
The study was performed at the Toxicology Department of Institut Pasteur de Lille for genotoxicity assays.

The computer applications used to acquire and derive data for this study included Excel® and Comet assay IV. These applications have been validated in the laboratory (Conformity certificates F-TOX-INF-025 and 024).

Otherwise, the computer application used to calculate mutation frequencies and percent RET was provided by the Manufacturer (Litron Laboratories Ltd) of the *In Vivo* MutaFlow Kits (*i.e.* kit used for the Pig-A test). This application was not validated in the laboratory.

Submitted by:

Study director **Dr. Sophie SIMAR**

17.2.17 

Date Signature

STUDY	***In Vivo* ERYTHROCYTES-BASED Pig-A GENE MUTATION ASSAY** **(Performed in Mouse somatic cells - Two sampling times)** **Combined to the** ***In Vivo* MAMMALIAN ALKALINE COMET ASSAY** **(Performed in Mouse Circulating blood cells - One sampling time)** **(Five treatments followed by 3 co-treatments)**
TEST ITEM	**ADN Telomeractives®**
SPONSOR	**HBN**

This report was reviewed and approved by:

Test Facility Management	**Dr. Fabrice NESSLANY** Head of Toxicology Department	16/02/2017	
		Date	Signature
Deputy Study Director	**Mrs. Gwendoline MORDACQ**		

***In Vivo* ERYTHROCYTES-BASED Pig-A GENE MUTATION ASSAY**
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SUMMARY

SPONSOR	:	**HBN**
TEST ITEM	:	**ADN Telomeractives®**
BATCH NUMBER	:	**N002**
STUDY LOCATION	:	**INSTITUT PASTEUR DE LILLE**
		Genetic Toxicology Laboratory
		1, rue du Professeur Calmette - B.P. 245
		59019 LILLE CEDEX FRANCE

THIS STUDY WAS CARRIED OUT IN COMPLIANCE WITH GOOD LABORATORY PRACTICE REGULATIONS

Study initiation date (date Study Director signed Study Plan):	27/09/2016
Main assay	
Treatments (with the test item alone):	17 to 21/10/2016
Co-treatments (test item +/- ENU)	24 to 26/10/2016
Sampling for Comet assay	26/10/2016
D31/32 sampling for Pig-A	23&24/11/2016
D44 sampling for Pig-A	07/12/2016
Experimental completion date:	21/12/2016
Study completion	17/02/2017

AIM

The evaluation of the protective potential, eg. fight against primary DNA damage and/or optimization of DNA repair capability, of the ADN Telomeractives® test sample was studied using two different endpoints: the measurement of mutant frequency by *in vivo* Erythrocytes-Based Pig-A Gene mutation assay and the evaluation of primary DNA damage by the *in vivo* Comet assay following the alkaline version (pH > 13) in circulating blood cells in mice treated with both the test item and the positive reference substance ethylnitrosourea (ENU).

CONCLUSION

The test item ADN Telomeractives® (batch N002), provided by HBN, was investigated for its protective potential against DNA damaging agent, eg. fight against primary DNA damage and/or optimization of DNA repair capability, by the means of the evaluation of primary DNA damage by *in vivo* Comet assay following the alkaline version (pH > 13) in circulating blood cells based on OECD Guideline (No. 489, 2014) and the *in vivo* Erythrocytes-Based Pig-A Gene mutation assay, in male OF1 mice.

Animals were pre-treated with the test item alone at dose levels of 550 and 55 mg/kg. Oral treatments were carried out once a day for 5 consecutive days, 24 hours apart. Then, after 2 days without any treatment, mice were treated thrice, 24-hours apart, with the test item at the 2 same dose levels. One hour after each treatment with the test item, animals were treated with either the DNA damaging agent ethylnitrosourea or its vehicle.

The validity criteria for the results were fulfilled. The study was thus considered as valid.

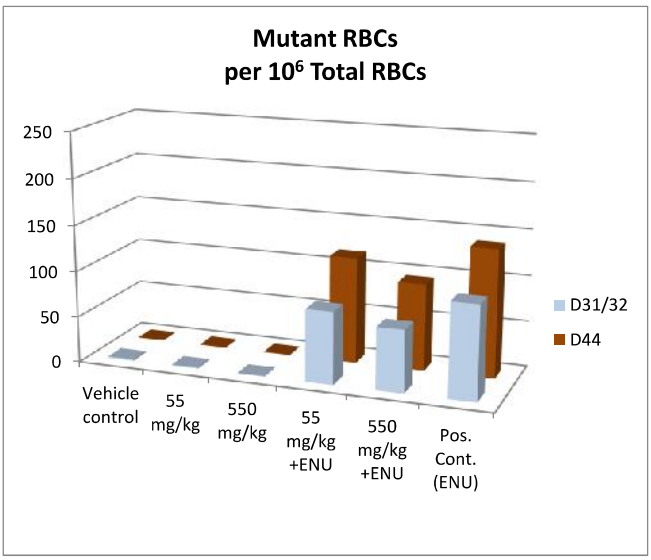
Under our experimental conditions, ADN Telomeractives® induced no mutagenic activity in circulating blood cells from OF1 male mice. Furthermore, the test item did not present DNA strand breaks and/or alkali-labile sites inducer activities toward the circulating blood cells from male OF1 mice,

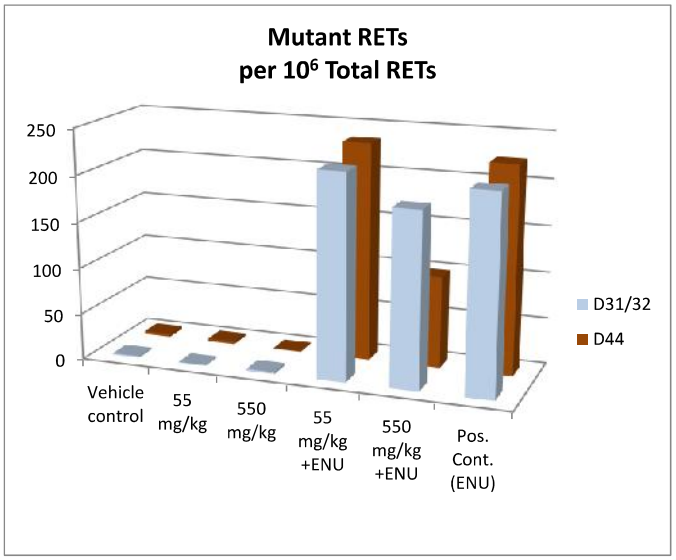
On the other hand, under these operating conditions, *in vivo*, ADN Telomeractives® decreased both DNA fragmentation and mutation frequency induced by ethylnitrosourea, a well-known potent mutagen/carcinogen. Therefore, ADN Telomeractives® is considered to have a protectant potential against primary DNA damage and mutation induced by a strong mutagenic substance ENU.

Figure 1

In Vivo **ERYTHROCYTES-BASED Pig-A GENE MUTATION ASSAY**
(Performed in Mouse somatic cells - Two sampling times)
Combined to the
In Vivo **MAMMALIAN ALKALINE COMET ASSAY**
(Performed in Mouse Circulating blood cells - One sampling time)
(Five treatments followed by 3 co-treatments)

Sponsor:	**HBN**	**Species:**	**Mouse**
Test item:	**ADN Telomeractives®**	**Strain:**	**OF1**
Vehicle:	**CMC at 0.5% in sterile water**	**Route:**	**oral**
		Volume:	**10 mL/kg***





** Phase I: 10 mL/kg/day (x5) - Phase II: 10 mL/kg/day (test item or vehicle control) + 10 mL/kg/day (x3) (ENU or sterile water)*