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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
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59019 LILLE CEDEX

SPONSOR
HBN
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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.

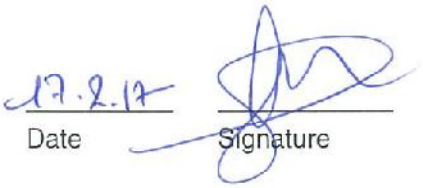
The study was performed at the Toxicology Department of Institut Pasteur de Lille for genotoxicity assays.

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**		

9.8 Interpretation of the results

A test item is considered clearly positive if:

- At least one of the treatment groups exhibits a statistically significant increase in the mean of medians of percentage of DNA in tail compared with the concurrent negative control,
- This increase is dose-related when evaluated with an appropriate trend test.

When these criteria are met, the test chemical is then considered able to induce DNA strand breakage in the tissues studied in this test system.

A test item is considered clearly negative if:

- none of the test concentrations exhibits a statistically significant increase compared with the concurrent negative control,
- there is no concentration-related increase when evaluated with an appropriate trend test,
- direct or indirect evidence supportive of exposure of, or toxicity to, the target tissue(s) has been demonstrated.

The test chemical is then considered unable to induce DNA strand breakage in the tissues studied in this test system.

This first statistical evaluation was applied for both **ADN Telomeractives®** and ENU (when used in co-treatment with sterile water) in order to assess their own mutagenicity when compared to the vehicle control.

Otherwise, in order to assess the possible "anti-mutagenic" activity, considering that **ADN Telomeractives®** is negative in this assay, the following criterion was used:

- no increase in the incidence primary DNA damage in the high and/or low group(s) co-treated is noted when compared to the relative groups treated with the test item alone or,
- on the contrary, if there is no significant decrease in the percentage of DNA in tail, **ADN Telomeractives®** should not be considered to have protective potential against the mutagenic reference substance test ENU.

9.9 Results for the Comet Assay

The assessment of the protectant potential of the test item **ADN Telomeractives®** against DNA damaging agent (ethyl nitrosourea – ENU), eg. fight against primary DNA damage and/or optimization of DNA repair capability, was investigated by using the evaluation of primary DNA damage by the *in vivo* Comet assay following the alkaline version (pH > 13) in circulating blood cells following the recommendation of the OECD guideline 489.

The evaluation of primary DNA damage by the *in vivo* Comet assay was done *ca.* 2 hours and 20 minutes after the last co-treatment.

The test results are summarized in Table 2 and Figure 2, Appendix No. 1.

The individual results are shown in Appendix No. 4a (Table 8).

The tables of data of individual values of percentage of DNA in Tail are presented in Appendix No. 4b.

In Appendix No. 4d are presented the results of statistical analysis.

No statistically significant increases in the median percentage of DNA in tail at the 2 analysed doses of 550 and 55 mg/kg (groups 3 and 4) **ADN Telomeractives®**, *vs.* the negative control (group 1). Indeed, the median percentages of DNA in tail were of 0.06 and 0.14% for the 55 and 550 mg/kg/day treatment groups, respectively, *vs*. 0.14 in the relative negative control group. A statistically significant decrease at the low dose of 55 mg/kg was noted without however any signification in terms of genotoxicity.

ADN Telomeractives® is thus **not genotoxic** under these experimental conditions.

Regarding co-treatment, significant decreases in the incidence of primary DNA damage in both the high and low co-treatment groups (groups 5 and 6) were noted when compared to the control group treated with ENU alone (group 2). Indeed, the median percentages of DNA in tail were of 5.87 and 3.93% for the 550 and 55 mg/kg/day treatment groups, respectively, *vs*. 8.24 in the ENU positive control group. The subsequent percentages of protective potential were of 29.3 and 53.2% at 550 and 55 mg/kg/day, respectively. The decrease was statistically significant at the low dose of 55 mg/kg co-treated group.

13 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 2

***In Vivo* ERYTHROCYTES-BASED Pig-A GENE MUTATION ASSAY**
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Combined to the
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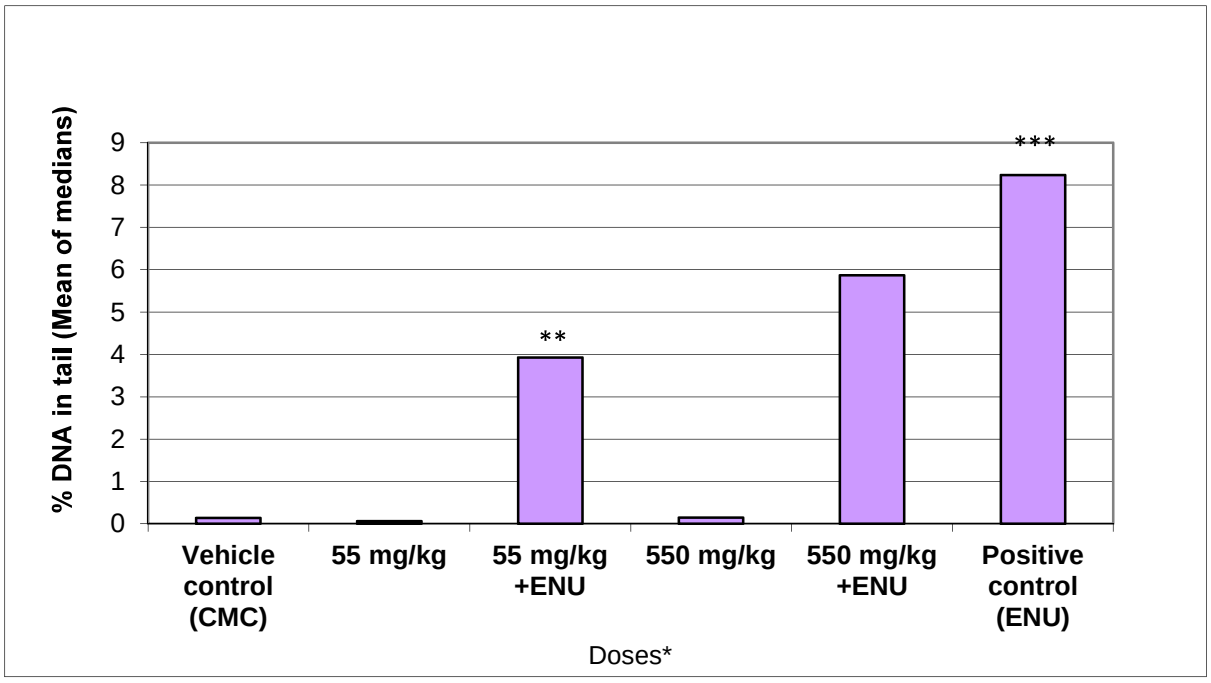
MEDIANS OF PERCENTAGES OF DNA IN TAIL PER GROUP

SPONSOR:	**HBN**	Species:	MICE
TEST ITEM:	**ADN Telomeractives®**	Strain:	OF1
VEHICLE:	CMC at 0.5% in sterile water	Sex:	MALE
DOSING VOLUME:	10 mL/kg*	Administration route:	oral
		Number of groups:	6 (with positive control group)
		Number of animals:	5 per group
		1st treatment:	17/10/2016+
ORGAN:	**BLOOD CELLS**	Last treatment:	26/10/2016

+: 24/10/2016 for positive control



ENU: Ethylnitrosourea

** 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)*

***: Statistically significant at p=1% when compared to ENU positive control*

****: Statistically significant at p=1% when compared to vehicle control*