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韓国化学融合試験研究院
2019年11月18日
試験責任者 Kim Ji-Sun

Final report

TGK-0021-15

TEPIC-VL

Mammalian erythrocyte micronucleus test of TEPIC-VL

KOREA TESTING & RESEARCH INSTITUTE

Choi Hyeongki

GLP statement

Study title : Mammalian erythrocyte micronucleus test of TEPIC-VL

Study number : TGK-0021-15

Sponsor

Name : NISSAN CHEMICAL INDUSTRIES, LTD.
Address : Kowa Hitohashi Building, 7-1, Kanda-nishiki-cho 3-chome, Chiyoda-ku, TOKYO 101-0054, JAPAN
Contact Number : Tel. +81-3-3296-8012 Fax. +81-3-3296-8360

Monitor

Name : LSI Medience Corporation
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Test facility

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Test facility Management : Kim Su-hyon
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This study was conducted under the supervision of the study director in compliance with the principles of Good Laboratory Practice.

1. Good Laboratory Practice (GLP)

1.1. OECD Principle of Good Laboratory Practice, ENV/MC/CHEM (98)17 (as revised in 1997)

2. Test regulation

2.1. OECD guidelines for the testing of chemicals, Section 4, TG. No. 474 'Mammalian Erythrocyte Micronucleus Test' (2014)

This study was performed by the alteration study plan, and the report provides a true and accurate record of the results obtained.

Study Director	Kim Ji-su, B.S	2015-11-18 Date
Test Facility Management	Kim Su-Hyon	2015-11-18 Date
	Kim Su-hyon, Ph.D.	

Quality assurance statement

Study title : Mammalian erythrocyte micronucleus test of TEPIC-VL

Study number : TGK-0021-15

Inspection phases	Inspections	Reports to study director	Reports to management
Draft study plan audit	2015-09-14	2015-09-15	2015-09-15
Study plan audit	2015-09-30	2015-09-30	2015-09-30
Preparation of the test substnace (1)	2015-10-06	2015-10-06	2015-10-06
Preparation of the test substnace (2)	2015-10-07	2015-10-07	2015-10-07
Test substance administration audit (1)	2015-10-07	2015-10-07	2015-10-07
Preparation of the test substnace (3)	2015-10-14	2015-10-14	2015-10-14
Test substance administration audit (2)	2015-10-14	2015-10-14	2015-10-15
Autopsy and slide preparation	2015-10-16	2015-10-16	2015-10-16
Slide Observation	2015-10-19	2015-10-19	2015-10-19
Raw data audit	2015-11-09	2015-11-09	2015-11-09
Draft final report audit	2015-11-09	2015-11-09	2015-11-09
Final report audit	2015-11-18	2015-11-18	2015-11-18

Inspections of the routine and repetitive procedures that constitute the study were carried out as a continuous process designed to encompass the major phases at or about the time this study was in progress.

This report has been audited by KTR Quality Assurance Unit, and is considered to be an accurate account of the raw data generated and of the procedures followed.

Inspections were accomplished as noted, and reported to the study director and management immediately following their completion. Based on these inspections and the review of the report, this study was conducted and reported in conformance with the Good Laboratory Practice regulations.

QA Person Jang Sung-yong, B.S. 2015-1-18 Date

Study staffs

Following staffs conducted in compliance with the KTR SOPs and the study plan of this study.

Study person	:	Kim Ji-yeon/B.S.
Chief of test substance preparation	:	Kim Pyeong-yeol/M.S.
Test substance administration	:	Kim Ji-su/B.S.
Specimen preparation	:	Kim Ji-yeon/B.S.
Observation	:	Kim Ji-su/B.S.
Data processing, statistics and final report drawing up	:	Kim Ji-su/B.S.

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1. Summary

To evaluate the genotoxicity of TEPIC-VL, a micronucleus test was performed using the bone marrow cells of specific pathogen free (SPF) male ICR mice.

On the basis of dose range-finding test, the highest dose of the main test was determined at 1500 mg/kg B.W.

In main test, After 1 week acclimation of 6-week old male mice, the test substance was orally administered twice at 24-hour intervals at a dose of 0, 187, 375, 750 and 1500 mg/kg B.W., and 5 animals per each group were used. The positive control was administered intraperitoneally once at dose of 70 mg/kg B.W.

The results of the test substance administration, dead animals was not confirmed. The number of micronucleated polychromatic erythrocyte (MNPCE) of polychromatic erythrocytes (PCEs) per a mouse was counted.

As a result, there was no increase of MNPCE at any dose of test substance compared to the negative control group, and also statistical significance was not observed.

Moreover, no statistical significance was observed in the value for the ratio of PCE to total erythrocytes [PCE/(PCE+NCE)] between the test substance-dosed group and negative control group. As expected, there was a statistical significance in number of MNPCEs in the positive control group.

Therefore, the test substance, TEPIC-VL, was determined not to induce an increased frequency of micronuclei in the bone marrow cells of male ICR mice under the present experimental condition.

2. Introduction

The test substance, TEPIC-VL, was evaluated for the potential to cause genotoxic changes by micronucleus test in bone marrow cells of male ICR mice.

ICR mice used in the test have been widely used for micronucleus test and were chosen because the accumulated data related to the micronucleus test were abundant to analyze the result of test.

This study was conducted in compliance with the methods described in the OECD guidelines for the testing of chemicals, Section 4, TG. No. 474 'Mammalian Erythrocyte Micronucleus Test' (2014).

This study was executed in accordance with the regulation for Institutional Animal Care and Use Committee at Health Care Research Laboratory in Korea Testing & Research Institute (KTR) based on Animal Protection Act. No. 13023 [enforcement, 2015-01-20] and Laboratory Animal Act. No. 11987 [enforcement, 2013-07-30].

2.1 Schedule of the study

Study initiation date	: 2015-09-30
Experiment starting date	: 2015-10-01
Dose range-finding test	
Animal reception and quarantine	: 2015-10-01
Animal classification	: 2015-10-06
Administration	: 2015-10-06 ~ 07
Administration periods	: 2015-10-06 ~ 07
Main test	
Animal reception and quarantine	: 2015-10-08
Animal classification	: 2015-10-14
Administration	: 2015-10-14 ~ 15
Administration periods	: 2015-10-14 ~ 15
Autopsy and slide preparation	: 2015-10-16
Experimental completion date	: 2015-10-28
Final report (Draft) preparation date	: 2015-11-06
Study completion date	: 2015-11-18

3. Materials & methods

3.1 Test substance

Name	: TEPIC-VL
Supplier	: NISSAN CHEMICAL INDUSTRIES, LTD.
KTR code	: TS-00300
LOT No.	: 150701
Purity	: 98 %
Appearance and Characteristics	:
Storage condition in study periods	: Room temperature [(15 - 25) ℃]

3.2 Control substance

3.2.1 Negative control (Vehicle)

Name (Synonym)	Manufacturer	LOT No.	Storage condition
Kolliphor® EL (Cremophor® EL)	Sigma-Aldrich Co.	BCBP4773V	Room temperature [(15 ~ 25)] ℃

3.2.1.1 Justification for selection of the negative control

In the preliminary test for the selection of the Vehicle, the test substance was at 200 mg/mL dissolved in Kolliphor® EL. No heat, discoloration or foaming was observed in the preparation using Kolliphor® EL. Based on this result, Kolliphor® EL was selected as the vehicle for the test substance and used as the negative control substance in this study.

3.2.2 Positive control

Name	: Cyclophosphamide monohydrate (CPA)
Supplier	: Sigma-Aldrich, Inc.
Cas No.	: 6055-19-2
Lot No.	: MKBS0021V
Purity	: 99.9 %
Storage condition	: Cold room temperature [(2 - 8) ℃]

3.2.2.1 Justification for selection of the positive control

The chemical that is described in OECD guideline was chosen as positive control substance.

3.3 Test system

3.3.1 Test system

Animal species (strain)	: CrljOri:CD1 (ICR), MOUSE, SPF
Supplier	: ORIENT BIO Co., Ltd. 8, Hwaaksan-ro 124beon-gil, Buk-myeon, Gapyeong-gun, Gyeonggi-do, Korea
Sex and animal number	: Dose range-finding test : male, 30 animals Main test : male, 33 animals
Age (At receipt / At administration age)	: 6 weeks old / 7 weeks old
Body weight (At receipt)	: Dose range-finding test (26.81 - 30.17) g Main test (27.36 - 31.38) g
Body weight (At administration) (1st/2nd)	: Dose range-finding test (32.13 - 34.94 / 31.71 - 35.27) g Main test (32.68 - 36.18 / 32.19 - 36.99) g

3.3.2 Choice of test system

The ICR/Mouse used in this study because it has been used widely in toxicity study including mammalian erythrocyte micronucleus test and a lot of comparative data have been accumulated.

3.3.3 Quarantine and acclimation

All animals was examined for quarantine at the time of receipt and be acclimatized to housing condition. The healthy animals was selected and used for the study based on general health conditions.

3.3.4 Identification

The animals was labeled on tail with permanent marker pen and identification cards attached to each cage for an individual discrimination.

3.3.5 Group assignment

After acclimation periods, animals was weighed and randomly assigned according to body weight average and standard deviation.

3.3.6 Remnant animal

Remnant animals was euthanized on autopsy and slide preparation date.

3.4 Animal Housing

3.4.1 Animal room Number

Quarantine and acclimation	: Quarantine room
Treatment and observation	: Room number 6

3.4.2 Environmental conditions

Temperature	:	(22 ± 3)℃ The actual measurement results Dose range-finding test : (21.9 - 22.3) ℃ Main test : (21.5 - 21.8) ℃
Relative humidity	:	(50 ± 20)% R.H. The actual measurement results Dose range-finding test : (53.5 - 54.7) % R.H. Main test : (53.8 - 54.8) % R.H.
Air exchange	:	(10 - 20) times/h
Light cycle	:	Light 12 h (08:00 - 20:00) Dark 12 h (20:00 - 08:00)
Illumination	:	(150 - 300) Lux
Cage style	:	Polysulfonate cage
Cage size	:	(180W × 300D × 140H) mm
Animal per cage	:	Less than 5 animals

The temperature and relative humidity were monitored automatically every half-hour by automatic instrument and other environmental condition measured periodically by SOP. There were no influenceable variations for study in environmental measurements.

3.4.2.1 Food and water

The animals fed Rodent Diet 20 5053 [Labdiet, USA] and given the R/O water *ad libitum*.

3.4.2.2 Contaminant analysis of food and water

The absence of contamination was confirmed with periodical analysis results report of manufacture. The water periodically was analyzed in accordance with SOP of Korea Testing & Research Institute.

3.4.2.3 Bedding

Bedding (Beta chip) was obtained from SILLIKER Inc(USA) and that was autoclaved at 121 ℃ for 20 min before use.

3.5 Preparation of the test substnace

Test material was prepared the day of administration.

(1) The test substance was weighed and put in the mortar.

(2) After grinding in the mortar, and suspended into a small amount of vehicle.

(3) The solution was added to the volumetric flask.

(4) Adjusted the final volume.

(5) Mix well and then captured in tubes .

(6) Weighing and all operations of the test substance was conducted under room

temperature and yellow light.

(7) Test substance solutions was stored in the dark and room temperature until the time of administration.

3.6 Analysis of test substance

After consulting with sponsor, analysis of the concentration verification, stability and homogeneity of the test substance and the prepared test substance were not performed in this test facility.

3.7 Preparation of the control solutions

3.7.1 Preparation of the positive control

It was prepared under yellow light on administration day.

(1) The CPA 35 mg was weighed.

(2) It was dissolved into 5 mL of sterile distilled water (7 mg/mL).

(3) It was stored under room temperature and yellow light.

3.8 Testing method

3.8.1 Administration

3.8.1.1 Route of administration

The oral administration was selected to dose the test substance, and positive control was administrated with single intraperitoneal injection.

3.8.1.2 Justification for selection of route of administration

This method is widely used in the micronucleus test. And the route of administration is described in OECD guideline was chosen.

3.8.1.3 frequency of administration

It was injected twice (24 hour interval). However, the positive control was administered one time on the last administration day.

3.8.1.4 Justification for selection of frequency of administration

This method is widely used in the micronucleus test. And the route of administration is described in OECD guideline was chosen.

3.8.1.5 Method of administration

The test substance was administrated twice to starved animals for 3 – 4 hours by oral gavage (Sonde), and positive control was administrated with a single intraperitoneal injection at the same time as final (2nd) administration day.

3.8.2 Determination of dose range and treatment group

3.8.2.1 Dose range-finding test

8 study groups consisted of 7 test groups, and 1 negative control group are selected, and each group contains 3 mice.

Sex	Group	Dose	Number of administration	Route of administration	Number of animals (Animal identification number)
Male	G1	Negative control	2	Oral	3 animals (1101 ~ 1103)
	G2	62.5 mg/kg/day	2	Oral	3 animals (1201 ~ 1203)
	G3	125 mg/kg/day	2	Oral	3 animals (1301 ~ 1303)
	G4	250 mg/kg/day	2	Oral	3 animals (1401 ~ 1403)
	G5	500 mg/kg/day	2	Oral	3 animals (1501 ~ 1503)
	G6	1000 mg/kg/day	2	Oral	3 animals (1601 ~ 1603)
	G7	1500 mg/kg/day	2	Oral	3 animals (1701 ~ 1703)
	G8	2000 mg/kg/day	2	Oral	3 animals (1801 ~ 1803)

3.8.2.2 Main test

6 study groups consisting of 4 test groups, 1 negative control group and 1 positive control group are selected, and 5 animals per each group are used. Dose levels in the main test are decided based on the result of the dose range-finding test, and the test substance is administrated twice at 24 hours intervals by oral gavage.

	Dose (mg/kg B.W.)	concentration (mg/mL)	Volume (mL/kg/times)	Times	Route	Numbers of animals
Negative control	0	0	10	2	Oral	5 (1101 ~ 1105)
Test substance	187	18.7	10	2	Oral	5 (1201 ~ 1205)
	375	37.5	10	2	Oral	5 (1301 ~ 1305)
	750	75	10	2	Oral	5 (1401 ~ 1405)
	1500	150	10	2	Oral	5 (1501 ~ 1505)
Positive control	70	7	10	1	intraperitoneal	5 (1601 ~ 1605)

3.8.3 Observations

3.8.3.1 Clinical sign

During the test, all animals were observed 1 or more times a day.

3.8.3.2 Measurement of animal's body weight

The body weight were measured at the time of animal receipt, grouping, before administration and autopsy.

3.8.4 Slide preparation and Observation

3.8.4.1 Time of specimen preparation

Specimens were prepared within about 24 hours from final administration.

3.8.4.2 Justification for selection of **Time of specimen preparation**

This method is widely used in the micronucleus test. And the Time of specimen preparation is described in OECD guideline was chosen.

3.8.4.3 Evaluation using animals

It was evaluated for there is no death animal test group.

3.8.4.4 Production of specimen

Specimen production was performed in the following way.

(1) After cervical dislocation, the femur was removed with as little blood as possible.

(2) The bone marrow was flushed with FBS (Fetal Bovine Serum).

(3) The cells was selected to the centrifuge tube and centrifuged at 1000 rpm for 5 min.

(4) Supernatant was discarded, and concentrated cells were suspended with a little fresh FBS.

(5) The suspended cells were smeared on a clean slide.

(6) The smeared slides were air-dried and thereafter fixed for 5 min in 99.9 % methanol.

3.8.4.5 Staining method

For the observation, 40 μg/mL acridine orange were dropped on the mathanol-fixed slides, and thereafter cover glass was placed on.

3.8.4.6 Observation of specimen

3.8.4.6.1 Observation method

The observation was done with blind method. The slides were observed under the fluorescent microscope at the magnification of over 400X.

3.8.4.6.2 Criteria for judgement

- Discrimination of Polychromatic Erythrocytes : Polychromatic erythrocytes are determined by appearance of orange-fluorescent light without nuclei.
- Discrimination of Nonchromatic Erythrocytes : Nonchromatic erythrocytes are determined by appearance of only their black shadows without fluorescent light.
- Criteria of micronucleus

  Size : From the smallest distinguishable one to the one as large as a half the diameter of erythrocytes.

  Shape : Mainly round and includes the form of donut shape, half moon shape and so on.

  Color : Same color with near cell nucleus; green fluorescent in acridine orange.

3.8.4.6.3 Observation of specimen

Within good smeared space, micronucleated polychromatic erythrocytes (MNPCE) were

counted in 4000 polychromatic erythrocytes (PCE) per animal. In addition, PCE frequency in 500 whole erythrocytes (PCE+NCE) were calculated.

3.9 Statistics

The following statistic analyses were done using a SPSS program (Ver. 19). The result of the statistical evaluation was regarded significantly when the *P* value was less than 0.05.

Test for differences of numbers of MNPCEs between treated and negative control group : Kruskal-Wallis' H-test.

Test for differences of numbers of MNPCEs between positive and negative control group : Mann-Whitnes's U-test.

Test for differences of PCE/(PCE+NCE) ratio between treated (Including positive control group) and negative control group : value of each data was subjected to ANOVA and Dunnett's test.

Test for differences of PCE/(PCE+NCE) ratio between treated (Including positive control group) and negative control group : value of each data was subjected to Student's t-test (Lovell, et. al. 1989).

For comparison of body weight of animals at the time of sacrifice, ANOVA and Dunnett's test were performed.

4. Amendments and deviations from the study plan

There were no amendments or deviations from the study plan of this study.

5. Records and Archives

All records which created during study period should be archived for 5 years after issuance of final report. After expiration of the storage period, additional storage determined by KTR' s SOPs.

5.1 Storage list

(1) Records on the study plan
(2) Records and raw data on the test substance
(3) Records and raw data on the test system
(4) Records on the observation and measurement
(5) Records on the communication with sponsors
(6) Records on the final report

5.2 Archive

Archive room (Ⅰ), (Ⅱ) of Health Care Research Laboratory, KTR.

6. Results

6.1 Dose range-finding test

(1) In the highest dose group(2000 mg/kg B.W.), there was a dead animals after treatment of test substance (Annex 1).

(2) Based on dose range-finding test, the highest dose of test substance in main test was determined at 1500 mg/kg B.W.

6.2 Result of main test

6.2.1 Weight

There was no statistical significance in body weights compared with the negative control

6.2.2 Clinical sign

In all groups of main test, there was no dead animal (Table 2, Appendix 2).

6.2.3 frequencies of MNPCE & ratio of PCEs to total erythrocytes

As a result of observation in 4000 PCEs, the frequencies of MNPCE were (0.14 ± 0.05) % in negative control, (0.15 ± 0.01) % in 375 mg/kg B.W., (0.20 ± 0.05) % in 750 mg/kg B.W., (0.16 ± 0.04) % in 1500 mg/kg B.W., and (7.46 ± 0.48) % in positive control. In other words, there was no statistical significance at any dose of the test substance. The positive control group showed statistical significance as anticipated ($P<0.01$).

The ratio of PCEs to total erythrocytes as an index of bone marrow toxicity was (61.66 ± 1.56) %, (61.16 ± 2.08) %, (59.81 ± 2.20) %, (57.53 ± 3.03) % and (37.50 ± 2.04) % in the same order as described above. There was no statistical significance shown at any dose of the test substance. The positive control group showed statistical significance as anticipated ($P<0.01$).

7. Discussion & conclusion

Our results showed that there was no increase of MNPCE at any dose of test substance compared to the negative control group, and also statistical significance was not observed. In addition, no statistical significance was observed in the value for the ratio of PCE to total erythrocytes [PCE/(PCE+NCE)] between the test substance-dosed group and negative control group.

Therefore, the test substance, TEPIC-VL, was determined not to induce an increased frequency of micronulei in the bone marrow cells of male ICR mice under the present experimental condition.

8. References

OECD guidelines for the testing of chemicals, Section 4, TG. No. 474 'Mammalian Erythrocyte Micronucleus Test' (2014).

OECD Principles of Good Laboratory Practice, ENV/MC/CHEM (98)17 (as revised in 1997).

Lovell, D.P., D. Anderson, R. Albanese, G.E. Amphlett, G. Clare, R. Ferguson, M. Richold, D.G. Papworth, and J.R.K. Savage (1989): Statistical analysis on in vivo cytogenetic assays, In : Statistical evaluation of mutagenicity test data (Kirkland, D.J. ed.), Cambridge University Press, Cambridge, U.K., pp. 184-232.

9. Tables

Table 1. Micronucleus test in ICR mice (Group summary)

Sex	Chemical treated	Dose (mg/kg B.W.)	No. of animal	MNPCE/2000PCEs (Mean±S.D., %)	PCE/(PCE+NCE) (Mean±S.D., %)
Male	Negative control	0	5	0.14 ± 0.05	61.66 ± 1.56
	Test substance	187	5	Not counted	
	Test substance	375	5	0.15 ± 0.01	61.16 ± 2.08
	Test substance	750	5	0.20 ± 0.05	59.81 ± 2.20
	Test substance	1500	5	0.16 ± 0.04	57.53 ± 3.03
	Positive control (CPA)	70	5	7.46 ± 0.48 *	37.50 ± 2.04 **

* $P<0.05$, ** $P<0.01$

MNPCE : PCE with one or more micronuclei

PCE : Polychromatic erythrocyte

NCE : Normochromatic erythrocyte

CPA : Cyclophosphamide monohydrate (positive control)

Table 2. Clinical signs and mortalities (Group summary)

Sex	Chemical treated	Dose (mg/kg B.W.)	Clinical signs	Mortality (dead / total)
Male	Negative control	0	N	0 % (0/5)[a]
	Test substance	187	N	0 % (0/5)[a]
	Test substance	375	N	0 % (0/5)[a]
	Test substance	750	N	0 % (0/5)[a]
	Test substance	1500	N	0 % (0/5)[a]
	Positive control (CPA)	70	N	0 % (0/5)[a]

[a] : No. of dead animals/No. of tested animals

CPA : Cyclophosphamide monohydrate

Table 3. Body weights of animals (Group summary)

Sex	Chemical treated	Dose (mg/kg B.W.)	Body weights (mean±S.D. g) at the time of Adiministration (No. of animal) 1st	Adiministration (No. of animal) 2nd	Sacrifice (No. of animal)
Male	Negative control	0	33.42 ± 2.17 (5)	34.43 ± 1.27 (5)	34.12 ± 1.15 (5)
	Test substance	187	34.44 ± 1.09 (5)	34.87 ± 0.79 (5)	33.80 ± 0.88 (5)
	Test substance	375	34.17 ± 1.27 (5)	34.08 ± 1.30 (5)	33.66 ± 1.45 (5)
	Test substance	750	34.59 ± 1.26 (5)	35.09 ± 1.91 (5)	34.46 ± 1.83 (5)
	Test substance	1500	34.62 ± 0.83 (5)	35.44 ± 0.75 (5)	34.90 ± 1.16 (5)
	Positive control (CPA)	70	35.04 ± 1.07 (5)	35.26 ± 0.95 (5)	34.26 ± 0.37 (5)

CPA : Cyclophosphamide monohydrate

10. Appendices

Appendix 1. Micronucleus test in ICR mice (Individual data, male)

Chemical treated	Dose (mg/kg B.W.)	Animal No.	PCE's	MNPCE	Frequency of MNPCE (%)	No. of PCE/NCE	PCE/ (PCE+NCE)
Negative control	0	1101	4000	7	0.18	385 / 259	59.78
		1102	4000	4	0.10	361 / 233	60.77
		1103	4000	8	0.20	378 / 215	63.74
		1104	4000	4	0.10	381 / 227	62.66
		1105	4000	5	0.13	363 / 229	61.32
Test substance	187	1201					
		1202					
		1203			Not counted		
		1204					
		1205					
Test substance	375	1301	4000	6	0.15	349 / 208	62.66
		1302	4000	6	0.15	399 / 286	58.25
		1303	4000	6	0.15	372 / 213	63.59
		1304	4000	5	0.13	345 / 226	60.42
		1305	4000	6	0.15	375 / 241	60.88
Test substance	750	1401	4000	8	0.20	412 / 315	56.67
		1402	4000	6	0.15	385 / 229	62.70
		1403	4000	11	0.28	412 / 285	59.11
		1404	4000	7	0.18	384 / 256	60.00
		1405	4000	7	0.18	381 / 248	60.57
Test substance	1500	1501	4000	7	0.18	491 / 296	62.39
		1502	4000	8	0.20	349 / 296	54.11
		1503	4000	4	0.10	356 / 274	56.51
		1504	4000	7	0.18	364 / 276	56.88
		1505	4000	6	0.15	354 / 259	57.75
Positive control (CPA)	70	1601	4000	315	7.88	213 / 349	37.90
		1602	4000	319	7.98	227 / 364	38.41
		1603	4000	299	7.48	205 / 374	35.41
		1604	4000	274	6.85	213 / 386	35.56
		1605	4000	285	7.13	235 / 349	40.24

MNPCE : PCE with one or more micronuclei

PCE : Polychromatic erythrocyte, NCE : Normochromatic erythrocyte

CPA : Cyclophosphamide monohydrate (Positive control)

Appendix 2. Clinical signs and mortalities (Individual data, male)

Chemical treated	Dose (mg/kg B.W.)	Animal No.	Clinical signs
Negative control	0	1101	Normal
		1102	Normal
		1103	Normal
		1104	Normal
		1105	Normal
Test substance	187	1201	Normal
		1202	Normal
		1203	Normal
		1204	Normal
		1205	Normal
Test substance	375	1301	Normal
		1302	Normal
		1303	Normal
		1304	Normal
		1305	Normal
Test substance	750	1401	Normal
		1402	Normal
		1403	Normal
		1404	Normal
		1405	Normal
Test substance	1500	1501	Normal
		1502	Normal
		1503	Normal
		1504	Normal
		1505	Normal
Positive control (CPA)	70	1601	Normal
		1602	Normal
		1603	Normal
		1604	Normal
		1605	Normal

CPA : Cyclophosphamide monohydrate

Appendix 3. Body weights of animals (Individual data, male)

Chemical treated	Dose (mg/kg B.W.)	Animal No.	Body weights (g) at the time of		
			Adiministration		Sacrifice
			1st	2nd	
Negative control	0	1101	30.03	32.91	32.83
		1102	34.87	35.81	35.54
		1103	32.97	33.41	33.11
		1104	33.56	34.50	34.24
		1105	35.65	35.51	34.89
Test substance	187	1201	34.39	35.19	34.60
		1202	33.35	33.94	34.05
		1203	36.04	35.97	32.38
		1204	33.56	34.29	33.58
		1205	34.88	34.98	34.39
Test substance	375	1301	32.87	32.66	32.07
		1302	36.13	36.18	35.96
		1303	33.66	33.67	32.96
		1304	33.52	33.69	33.46
		1305	34.69	34.19	33.83
Test substance	750	1401	35.75	36.99	36.61
		1402	34.40	35.12	34.35
		1403	34.40	34.57	33.95
		1404	35.72	36.60	35.60
		1405	32.68	32.19	31.79
Test substance	1500	1501	35.02	35.66	35.59
		1502	35.43	36.62	36.58
		1503	35.09	35.30	34.40
		1504	34.22	34.83	34.04
		1505	33.36	34.81	33.87
Positive control (CPA)	70	1601	36.18	35.60	34.53
		1602	35.11	35.40	34.17
		1603	33.29	33.63	33.65
		1604	35.49	35.51	34.38
		1605	35.14	36.15	34.55

CPA : Cyclophosphamide monohydrate

11. Annexes

Annex 1. Mortality of dose range-finding test

Sex	Chemical treated	Dose (mg/kg B.W.)	Mortality (dead / total)
Male	Negative control	0	0 % (0 / 3)
	Test substance	62.5	0 % (0 / 3)
	Test substance	125	0 % (0 / 3)
	Test substance	250	0 % (0 / 3)
	Test substance	500	0 % (0 / 3)
	Test substance	1000	0 % (0 / 3)
	Test substance	1500	0 % (0 / 3)
	Test substance	2000	33.3 % (1 / 3)

Annex 2. Historical background data

[2014 - 2015]

	Micronucleus				PCE/(PCE+NCE) (%)	
	Negative control[a]		CPA[b]			
	MNPCE	Frequency of MNPCE(%)	MNPCE	Frequency of MNPCE(%)	Negative control	CPA
Mean[c]	1.47	0.07	90.50	5.81	52.31	47.33
SD	0.84	0.06	23.79	1.55	2.34	3.37
Minimum	0	0	39	2.68	49.30	36.26
Maximum	5	0.24	154	7.51	63.10	51.59

(updated March 09, 2015)

[a] Calculated on the basis of data from 10 experiments (Number of animals = 50)

[b] Calculated on the basis of data from 10 experiments (CPA) (Number of animals = 50)

[c] MNPCE/PCE

Negative control

(distilled water, 0.5 % CMC-Na Sol.,1 % MC Sol., Corn oil, or vehicle supplied by Sponsor)

CPA : Cyclophosphamide monohydrate

Annex 3. Information of test substance (Submitted by sponsor)

試験物質情報記録紙

* 正確で信頼性のある試験の進行のために試験物質に対する内容を正確に作成してください。
* 物質が2種以上の場合、物質毎に記録紙を作成してください。

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作成日 : 2015年 8月 27日	作成者 : Mikio Kasai (署名) Mikio Kasai
試 験 物 質 (Test substance)	
試験物質名 (Name of test substance)	TEPIC-VL
分類 (Classification)	□健康機能食品 □農薬 □医薬品 ■化学物質 □化粧品 □その他
供給源 (Supplier)	NISSAN CHEMICAL INDUSTRIES, LTD.
製造日 (Manufacturing date)	2015年7月1日
提供量 (Delivery amount)	■実重量(Net): 20g □容器含む(Gross): g (g × □個 □瓶)
Cas No.	91403-64-4
ロット番号 (LOT No.)	150701
試験期間中保管条件 (Storage condition)	■室温(1~30℃) □常温(15~25℃) □冷蔵(2~8℃) □冷凍(-15~-25℃) □その他 ( )
有効期間 (Expiration date)	2020年 7月 1日 (製造後 5年)
外観及び性状 (Physical description)	無色透明
純度 (Purity)	98 %
分子式(分子量) (Molecular formular or weight)	$C_{18}H_{27}N_3O_6$ (381.4)
比重 (Specific gravity)	-
pH	-
オクタノール分配係数 (KOW)	1.6
水溶解度 (Solubility in water)	情報なし
残余試験物質処理方法 (Treatment after the end of study)	□返却 ■廃棄 (※GLP上の保管試料は除外)
取扱 / 廃棄時注意事項 (Caution in handling or disposal)	■MSDS参照 □無 ■その他 (ゴム手袋及びマスク着用)

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(Continued)

Annex 3. Information of test substance (Submitted by sponsor)

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臨床予定経路 (Route of clinic)	□経口 □経皮 □静脈 □その他( )	臨床予定用量 (Dose cono. of clinic)	□ (mg/kg B.W) □ (mL/kg B.W)
運送方法 (Delivery method)	□郵便 □直接伝達 ■その他 (国際運送)		
運送条件 (Delivery condition)	■室温(1~30℃) □常温(15~25℃) □冷蔵(2~8℃) □冷凍(-15~-25℃) □その他(遮光、紫外線を避けること)		
発送(予定)日 (Delivery date)	2015年 8月 31日		
試験物質調剤 (Preparation of the dosing solution)			
不経済 / 溶解度 (Vehicle and solubility)	□注射用水 □生理食塩水 ■DMSO ■DMF □ワセリン □エタノール □アセトン □植物オイル (Corn oil/Olive oil) □( )% CMC □( )% MC □その他( )		
安定性 (Stability)	加水分解性あり	均質性 (Homogeneity)	□溶液提供 □無 □その他( )
調剤物保管条件 (Storage condition of dosing solution)	■室温(1~30℃) □常温(15~25℃) □冷蔵(2~8℃) □冷凍(-15~-25℃) □その他(遮光、紫外線を避けること)		
調剤方法 (Preparation method)	□添付資料参考 □E-mail等で伝送 □その他( )		
注意事項 (caution)	□遮光 ■密閉 □除湿 □その他		
調剤物分析 (Analysis of the dosing solution)			
調剤物分析必要 (Analysis of the dosing solution in the study)	□必要 □不必要(調剤物分析成績書提出、試験物質CoAに代替) □その他 ( )		
分析法 (Analytical method)	□提供 (検出限界:) □分析法開発必要		
標準物質 (Standard material)	□提供 □試験物質に代替		
分析機器 (Analytical instrument)	□LC □GC □その他( )		
検出器 (Detector)	□UVD □FLD □PDA □MSD □ECD □NPD		
添付資料 (Attachment)	■試験物質の成績書(Certificate of Analysis) ■MSDS □試験物質安定性資料 □薬効薬理資料 □毒性試験試料 □他機関報告書 □試験物質参考資料 □参考文献 □試験物質調剤方法 □分析法 □標準物質 □その他( )		
備考 (Remark)	(懸案希望事項又はその他情報等に対して記述してください。)		

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(End)

Annex 4. Certificate of analysis (Submitted by sponsor)

2015/8/31

Quality Certificate

NISSAN CHEMICAL INDUSTRIES, LTD.
Planning & Development Department,
Chemicals Division

sample	TEPIC-VL				
Lot No.	150701	volume	20 g x 1	shipping date	

	unit	specification (temporary)	analysis value
Epoxy equivalent weight	g/eq	125-145	130
Viscosity (25 degrees)	mPa·s	6,000-8,000	7,310
Purity "	%	-	98

Annex 5. Receipt of test substance

시 험 물 질 수 령 기 록 지

수 신	LSI Medience Corporation			
발 신	주 소	헬스케어연구소 (519-955) 전라남도 화순군 화순읍 산단길 12-63		
연락처	E-mail	kpy123@ktr.or.kr		
	Telephone	061-370-7854	Fax	061-370-7777

귀사의 발전을 기원합니다.
귀사에서 의뢰하신 시험에 대한 시험물질을 아래와 같이 수령하였음을 알려드립니다. 만일 귀사에서 발송하신 시험물질 내역과 상이한 경우 연락주시기 바랍니다.

시험물질명 (Name of test substance)	TEPIC-VL
Lot No.	150701
입수량 (용기포함) (Receipt amount)	((54.472 g) × 1 (□개 ■병))
입수일 (Receipt date)	2015-09-07
보관조건 (Storage condition)	상온 (15℃ ~ 25℃)

위 시험물질의 수령을 확인함

조제분석책임자 :