RUSSIAN FEDERATION MINISTRY OF HEALTH AND SOCIAL DEVELOPMENT

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FEDERAL MEDICO-BIOLOGICAL AGENCY OF RUSSIA
(SFES RCT&HRB FMBA RF)

Approved:

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prof. N.R. Dyadishchev, Dr. Sci (Med)
Director of SFES RCT&HRB
"____"_____2011

REPORT

Assessment of mutagenic effects of Killevir-16 substance at peroral administration by chromosome aberrations assay on murine bone marrow cells

(Contract ZT 22/2011)

Study Director:

Larisa V. Mikhina, PhD

Serpukhov– 2011

RESPONSIBLE PERSONNEL

L.V. Mikhina, PhD	Principal Scientist
A.V. Tretiakova	Senior Researcher
L.A. Eremenko	Researcher
S.V. Melnikova	Junior Researcher
E.N. Sokolova	Junior Researcher
Z.M. Dmitrieva	Laboratory Assistant

ABBREVIATIONS

DMSO	–	dimethyl sulfoxide
TD	–	therapeutic dose

SUMMARY

Report: 14 pages, 2 tables, 5 references, 1 annex

Key words: *pharmacological substance, mutagenic effect, preclinical trials, chromosomal aberrations, Killevir-16 substance*

Mutagenic effect of pharmacological substance Killevir-16 was assessed using chromosome aberration assay on C_{57}Bl/6 mouse bone marrow cells after 4-repeated peroral administration in equitherapeutic dose (1 TD) and 10fold TD.

Doses for animals were calculated by correlation of human and mouse body surface areas: 1 TD for humans – 40 mg, 1 TD for mice =0.13 mg/animal; 10 TD=1.33 mg/animal. Number of animals in experimental groups – 5 per sex.

The test substance was administered in animals in the form of suspension in 1% starch gel. The mice from negative control received a 4-repeated peroral dose of starch gel, positive control – a single intraperitoneal dose (20 mg/kg) of cyclophosphamide.

The studies conducted have not revealed a reliable increase in the numbers of cells with chromosome aberrations in male and female mice exposed to the test substance in doses up to 10 TD; the number of cells with chromosome aberrations was not higher than 4%. Qualitative characteristics of cell damages caused by the test substance did not change: the majority of alterations observed were chromatid-type aberrations (end deletions); no cells with multiple aberrations were found.

Therefore, Killevir-16 did not have mutagenic effects on somatic cells at 4-repeated peroral administration in doses 0.13 mg/animal (equitherapeutic dose) and 1.33 mg/animal (the doses were calculated by correlation of human and mouse body surface areas based on a daily therapeutic dose for humans – 40 mg).

From the data obtained it follows that Killevir-16 substance in a daily peroral dose of 40 mg for humans does not have mutagenic effect.

CONTENTS

INTRODUCTION

Mutagenic effect of pharmacological substance Killevir-16 was assessed using chromosome aberration assay on C_{57}Bl/6 mouse bone marrow cells at 4-repeated peroral exposure.

Mutagenic activity of the test substance at a single intraperitoneal exposure in two doses: 1/5 LD_{50} and in the dose equivalent to a daily therapeutic dose for humans (TD) (calculated for experimental animals based on body surface area) was studied earlier.

The work was performed in compliance with Methodical recommendations of Pharmacological Committee of the Ministry of Health of the Russian Federation "Assessment of mutagenic properties of pharmaceuticals" [1].

1. **MATERIALS AND METHODS**

The experiments were conducted in accordance with the study protocol (Annex 1, study code L0611, substance code 0611) designed based on Methodical recommendations [1].

Test substance

Substance code: 0611

Name: Killevir-16

Manufacturer: ZAO Intelpharm

Molecular formula: $C_{60}(NH(CH2)_5COOH)_n$, where n=4-6

Molecular mass: 1500

pH: 5.2

Description: amorphous powder, brown or dark brown

Decomposition temperature without melting: 400-450 °C

Admixtures:

Amino-caproic acid: 3-3.5 %

Chlorides: ≤0.2%

Total ash: ≤0.5%

Hard metals: ≤0.001%

Residual vehicles:

1, 2- dichlorbenzene ≤0.032%

Nitrogen: 5.0-5.4%

Elemental analysis:

%C 69.52

%H 4.82

%N 5.20

%Cl no

Solubility:

- freely soluble in DMSO;
- soluble in dimethyl formamide (10 mg of substance in 10 ml of DMF);
- soluble in ice acetic acid (1 mg/10 ml);
- almost insoluble in water, 95% ethyl alcohol, and 1,2-dichlorbenzene

Batch #54, produced December 22, 2009

Shelf life – 5 years

Storage conditions: dry, protected from light, temperature ≤30°C.

Control substances

Negative control: test substance vehicle for peroral administration

Name	1% starch gel
Code	C^-

Positive control: a substance with mutagenic activity

Name	cyclophosphamide
Code	C^+

Preparation of substances for administering in animals

The test substance was administered in animals in the form of suspension in 1% starch gel. Before preparing a suspension the substance was ground in a mortar to obtain a homogenous powder.

Positive control – a solution based on physiological saline.

Pharmaceutical group prepared working solutions of test and control substances in aseptic conditions prior to dosing.

Animals

Species: mice

Strain: C_{57}Bl/6

Source: Animal Nursery of SCBMT RAMS, "Andreevka" branch, Solnechnogorsky district, Moscow region

Weight before study: 18-22 g

Number, sex: 25 male and 20 female mice (including reserve)

Identification: ear tags

Preparation of metaphase chromosomes

In order to suppress formation of achromatic spindle at cell deletion and accumulate metaphase material, in mice a single intraperitoneal dose of 0.025% colchicine in volume 0.25 ml was administered 2.5 hours before euthanasia.

After CO_2 euthanasia from animals thigh-bones were taken out and placed in vials with physiological saline.

Cytogenetic preparations were prepared by routine method (Preston R.J. et al. [4]). Bone marrow was washed off from thigh-bones with physiological saline warmed up to 37^0C. The obtained suspension of bone marrow cells was centrifuged (5 min, 1000-1200 rpm); the

supernatant was removed. Sediment cells were re-suspended in hypotonic solution of potassium chloride (0.56%), incubated for 10 min at 37^0C and precipitated by centrifuging (5 min, 1000 rpm). Supernatant was removed, and sediment cells were fixed by cooled mixture of alcohol-acetic acid (3:1). After fixation, cell suspensions were placed on wet cooled object glasses, dried on a burner and dyed with 0.5% gencian violet during 30 min. The obtained cytological preparations (2 glasses for every animal) were coded and examined under immersion system of light microscope.

Analysis of cytological preparations

Routine cytogenetic analysis was conducted according to recommendations of Scott D. et al. [5]. For the analysis, rounded metaphase plates without chromosome overlaying (modal number 40) were selected. The number of single and pair fragments, as well as chromatid and chromosomal exchanges and the number of cells with multiple chromosomal damages (over 5 per cell) were counted.

From each animal 100 metaphases were taken for analysis. The percentage of damaged cells was calculated for each animal and for the whole group. Achromatic gaps were not considered.

Statistical data processing

Statistical processing of experimental data was performed using Student's criterion [2].

2. STUDY RESULTS

Mutagenic effect of pharmacological substance Killevir-16 was assessed using chromosome aberration assay on C_{57}Bl/6 mouse bone marrow cells following 4-repeated administration of peroral equitherapeutic dose (1 TD) and 10fold TD.

Doses for animals were calculated by correlation of human and mouse body surface areas: 1 TD for humans – 40 mg, 1 TD for mice =0.13 mg/animal; 10 TD=1.33 mg/animal.

The test substance was administered in animals in the form of suspension in 1% starch gel. The mice from negative control received a 4-repeated peroral dose of starch gel, positive control – a single intraperitoneal dose of cyclophosphamide (20 mg/kg).

The experiments were conducted on mice of both sexes; the number of animals in experimental and control groups – 5.

Results of assessment of the test substance mutagenic effects at 4-repeated peroral administration are given in Tables 1-2. Following exposure to the test substance in two tested doses, in male and female mice no statistically reliable increase in the number of cells with structural damages of chromosomes compared to negative control was noted.

The character of damages in chromosomes of experimental animals did not differ from negative control: the majority of alterations were chromatid-type aberrations (end deletions). No cells with multiple deletions were found in animals treated with the test substance and in groups of negative control. The number of gaps in treated groups and in negative control was not higher than 2% (data are not included in Tables 1-2). In group of positive control after a dose of cyclophosphamide possessing mutagenic activity (20 mg/kg) the number of damaged cells was 13.40± 0.81%; the main damages were chromatid-type aberrations.

Table 1

Data from genetic screening of C_{57}Bl/6 male mice bone marrow cells at 4-repeated peroral administration of Killevir-16

Substance, dose	Mouse #	Number of tested cells	Number of cells with single aberrations	Number of aberrations			Number of cells with multiple aberrations	Number of damaged cells (%)	Number of aberrations per cell
				Single fragments	Pair fragments	Exchanges			
Killevir-16 0.13 mg per animal	1	100	1	1	0	0	0	1	0.01
	2	100	0	0	0	0	0	0	0.00
	3	100	1	1	0	0	0	1	0.01
	4	100	1	0	1	0	0	1	0.01
	5	100	0	0	0	0	0	0	0.00
	Total:	**500**	**3**	**2**	**1**	**0**	**0**	**0.60 ± 0.24**	**0.006 ± 0.002**
Killevir-16 1.33 mg per animal	11	100	0	0	0	0	0	0	0.00
	12	100	0	0	0	0	0	0	0.00
	13	100	2	2	0	0	0	2	0.02
	14	100	1	1	0	0	0	1	0.01
	15	100	1	1	0	0	0	1	0.01
	Total:	**500**	**4**	**4**	**0**	**0**	**0**	**0.80 ± 0.37**	**0.008 ± 0.004**
Negative control	26	100	1	1	0	0	0	1	0.01
	27	100	0	0	0	0	0	0	0.00
	28	100	2	2	0	0	0	2	0.02
	29	100	0	0	0	0	0	0	0.00
	30	100	1	0	1	0	0	1	0.01
	Total:	**500**	**4**	**3**	**1**	**0**	**0**	**0.80 ± 0.37**	**0.008 ± 0.004**
Positive control (cyclophosphamide) 20 mg/kg	21	100	13	15	4	0	2	15	0.19
	22	100	10	14	1	0	2	12	0.15
	23	100	14	8	2	0	0	14	0.10
	24	100	11	15	3	0	0	11	0.18
	25	100	15	18	2	0	0	15	0.20
	Total:	**500**	**63**	**70**	**12**	**0**	**4**	**13.40 ± 0.81**	**0.16 ± 0.018**

Table 2

Data from genetic screening of C_{57}Bl/6 female mice bone marrow cells at 4-repeated peroral administration of Killevir-16

Substance, dose	Mouse #	Number of tested cells	Number of cells with single aberrations	Number of aberrations			Number of cells with multiple aberrations	Number of damaged cells (%)	Number of aberrations per cell
				Single fragments	Pair fragments	Exchanges			
Killevir-16 0.13 mg per animal	6	100	0	0	0	0	0	0	0.00
	7	100	0	0	0	0	0	0	0.00
	8	100	1	2	0	0	0	1	0.02
	9	100	1	1	0	0	0	1	0.01
	10	100	0	0	0	0	0	0	0.00
	Total:	**500**	**2**	**3**	**0**	**0**	**0**	**0.40 ± 0.24**	**0.006 ± 0.004**
Killevir-16 1.33 mg per animal	16	100	1	1	0	0	0	1	0.01
	17	100	1	1	0	0	0	1	0.01
	18	100	0	0	0	0	0	0	0.00
	19	100	2	2	0	0	0	2	0.02
	20	100	0	0	0	0	0	0	0.00
	Total:	**500**	**4**	**4**	**0**	**0**	**0**	**0.80 ± 0.37**	**0.008 ± 0.004**
Negative control	31	100	1	1	0	0	0	1	0.01
	32	100	1	0	1	0	0	1	0.01
	33	100	2	2	0	0	0	2	0.02
	34	100	1	2	0	0	0	1	0.02
	35	100	0	0	0	0	0	0	0.00
	Total:	**500**	**5**	**5**	**1**	**0**	**0**	**1.00 ± 0.32**	**0.012 ± 0.004**

Microscopic analysis of the obtained cytological preparations did not reveal the increase in the number of cells with chromosome aberrations in bone marrow cells of experimental animals. The number of aberrations per cell did not differ from negative control. Killevir-16 did not have mutagenic effects on somatic cells of experimental mice at a 4-repeated peroral administration in doses up to 1.33 mg/animal.

CONCLUSION

Mutagenic effect of pharmacological substance Killevir-16 was assessed using chromosome aberration assay on C_{57}Bl/6 mouse bone marrow cells after 4-repeated peroral administration in equitherapeutic dose (1 TD) and 10 TD. Doses for animals were calculated by correlation of human and mouse body surface areas: 1 TD for humans – 40 mg, 1 TD for mice =0.13 mg/animal; 10 TD=1.33 mg/animal. Number of animals in experimental groups – 5 per sex.

The test substance was administered in animals in the form of suspension in 1% starch gel. The mice from negative control received a 4-repeated peroral dose of starch gel, positive control – a single intraperitoneal dose (20 mg/kg) of cyclophosphamide.

The studies conducted have not revealed a reliable increase in the numbers of cells with chromosome aberrations in male and female mice exposed to the test substance in doses up to 10 TD; the number of cells with chromosome aberrations was not higher than 4%. Qualitative characteristics of cell damages caused by the test substance did not change: the majority of alterations observed were chromatid-type aberrations (end deletions); no cells with multiple aberrations were found.

Therefore, Killevir-16 did not have mutagenic effects on somatic cells at 4-repeated peroral administration in doses 0.13mg/animal (equitherapeutic dose) and 1.33 mg/animal (the doses were calculated by correlation of human and mouse body surface areas based on a daily therapeutic dose for humans – 40 mg).

REFERENCES

1. Guidance on mutagenicity assessment of pharmaceuticals /Guidance on experimental (preclinical) study of new pharmaceuticals. Ed. by prof. R.U. Khabriev – M.: Federal State Enterprise “Research Center for Pharmaceutical Expertise”. – 2005. – p. 100 –122.
2. Methodical guidance on statistical treatment of preclinical trials data” /Guidance on experimental (preclinical) study of new pharmaceuticals. Ed. by prof. R.U. Khabriev – 2nd edition, corrected and amended; ad. – M.: Federal State Enterprise “Research Center for Pharmaceutical Expertise”, 2005. P.774 – 826.
3. Sanitary rules on construction, equipment and maintenance of experimental-biological clinics (vivariums). – Moscow. – 1973.
4. Preston R.J., Dean B.J., Galloway S., Holden, H., McFee A.F., and Shelby M. Mammalian *in vivo* Cytogenetic Assays: Analysis of Chromosome Aberrations in Bone marrow Cells //Mutation Research. – 1987. – №189. – C. 157-165.
5. Scott D., Danford N.D., Dean B.J. et al. Chromosome aberration assay in mammalian cells in vitro. // Report of the UKEMS Sub-Committee on Guidelines for Mutagenicity Testing (Ed. Dean B.J. United Kingdom Environmental Mutagen Society, Swnsea). – 1983. – C. 43-64.

ANNEX 1

STUDY PROTOCOL

Assessment of mutagenic effects of Killevir-16 substance by chromosome aberrations assay on murine bone marrow cells at peroral administration

Study code: L0611

Test substance code: 0611

Customer: ZAO Intelpharm

Pushkin str. 36, Nizhegorodskaya region, Chkalovsk, 606540, Russia

Fax: +7 8-831-433-00-03

Tel.: +7 8-831-430-02-06, 8-831-430-38-57

e-mail: info@intelpharm.ru

Testing facility:

State Federal Enterprise for Science "Research Centre for Toxicology and Hygienic Regulation of Biopreparations" (RCT&HRB), Federal Medico-Biological Agency
Bld. 102A Lenin str., Serpukhov, Moscow region, 142253, Russia
tel/fax: (4967) 39-97-38
toxic@online.stack.net

Study Director:
Larisa V. Mikhina, PhD
Head of Immunological Laboratory
tel/fax: (4967) 70-54-84, 39-97-38
toxic@online.stack.net

1. Key dates

Planned study initiation date: April 18, 2011
Planned first dose date: April 25, 2011
Planned dosing completion date: August 15, 2011
Planned study completion date: September 23, 2011

2. Responsible personnel:

- **Administration of test substances, clinical observations**

S.V. Melnikova, Junior Researcher, Immunological Laboratory,
E.N. Sokolova, Junior Researcher, Immunological Laboratory

- **Necropsy**

S.V. Melnikova

- **Cytological preparations**

E.N. Sokolova

- **Cytogenetic analysis**

Larisa V. Mikhina, PhD, Head of Immunological Laboratory

- **Animal care and veterinary control**

I.E. Selivanova, Head of Vivarium

- **Procurement and accountability of the test substances**

L.A. Eremenko, researcher, Division for Analytical Chemistry and Radiobiology, Head of Pharmaceutical Group

- **Quality Assurance**

V.V. Kapranov, Head of QA Laboratory

3. Study Objective

Detection and quantitative assessment of potential mutagenic activity of Killevir-16 substance for warm-blooded animals at peroral administration using chromosome aberration assay in murine bone marrow cells.

4. Guidelines

The study will be guided by the following documents:

1. "Rules for Laboratory Practice" (Order of Russian Ministry of Health and Social Development #708n of August 23, 2010);

2. GOST P 53434-2009. GLP principles. M.: Standartinform.- 2010. – 12p.

The study design was made up based on "Methodical guidance on allergenicity assessment of pharmaceuticals" /Guidance on experimental (preclinical) study of new pharmaceuticals. Ed. by prof. R.U. Khabriev. – M.: OAO "Medicina Publishing House", 2005. p.100 – 104.

All procedures within the study will be performed in accordance with the approved written study protocol and Standard operating procedures (SOPs).

4.1. Humane treatment and use of test animals

Animals will be handled and used in compliance with:

- RCT&HRB SOPs on animal care and maintenance,
- Statutory documentation of RCT&HRN Bioethics Committee,
- Sanitary rules approved by the Ministry of Health of the Russian Federation on 06.07.73 on construction, equipping and maintenance of experimental biological clinics (vivariums).

A veterinary protocol will be written and submitted to the Bioethics Committee of the RCT&HRB for expert assessment and approval. Laboratory animals for the studies will be purchased after approval of the veterinary protocol.

In the studies only the personnel who have appropriate qualification and skills will be involved. During the study all manipulations with animals will comply with the procedures of the approved protocol.

The study is included in complex nonclinical studies mandatory for the product registration on the territory of the Russian Federation. The information obtained in the course of the study will not repeat the earlier obtained study results.

4.2. Quality assurance

Quality Assurance Laboratory conducts audit of the principal phases of the study for consistency of procedures with the approved protocol, reliability of the obtained data and correctness of documentation.

5. Test and control substances

5.1. Test substance

Substance code : 0611

Name: Killevir-16

Manufacturer: ZAO Intelpharm

Molecular formula: $C_{60}(NH(CH2)_5COOH)_n$, where n=4-6

Molecular mass: 1500

pH: 5.2

Description: amorphous powder, brown or dark brown

Decomposition temperature without melting: 400-450 °C

Admixtures:

Amino-caproic acid: 3-3.5 %

Chlorides: ≤0.2%

Total ash: ≤0.5%

Hard metals: ≤0.001%

Residual vehicles:

1, 2- dichlorbenzene ≤0.032%

Nitrogen: 5.0-5.4%

Elemental analysis:

%C 69.52

%H 4.82

%N 5.20

%Cl no

Solubility:

- freely soluble in DMSO;
- soluble in dimethyl formamide (10 mg of substance in 10 ml of DMF);
- soluble in ice acetic acid (1 mg/10 ml);
- almost insoluble in water, 95% ethyl alcohol, and 1,2-dichlorbenzene

Batch #54, produced December 22, 2009

Shelf life – 5 years

Storage conditions: dry, protected from light, temperature ≤30°C.

5.2. Control substances

Negative control: test substance vehicle for peroral administration

Name	1% starch gel
Code	C^-

Positive control: a substance with mutagenic activity

Name	cyclophosphamide
Code	C^+

5.3. Composition and stability

The composition and stability of the test substance are the responsibility of the customer**.** Results of a component analysis are submitted to the Study Director and stored with raw data.

5.4. Substance specimens

Pharmaceutical group maintains records on procurement and accountability of the test substances and maintains specimens of each lot of test and control substances in allocated room under proper storage conditions.

Reserve specimens of test substance are stored with the other study materials.

5.5. Preparation of substances for administering in animals

The test substance suspension in 1% starch gel will be administered in animals. To prepare a suspension the substance will be ground in a mortar to homogenous powder.

Positive control – a solution based on physiological saline.

Working solutions will be prepared in aseptic conditions by pharmaceutical group prior to dosing.

6. Animals

Species: C_{57}Bl/6 mice

Source: Animal Nursery of SCBMT RAMS, "Andreevka" branch, Solnechnogorsky district, Moscow region

Weight before studies: 8-22g

Number, sex: 25 male, 20 female (including reserve)

Identification: ear tags.

6.1. Animal care

Animals will have free access to feed and water. Animal care and maintenance will comply with guidance documents. All routine procedures will be performed in accordance with the SOPs of the RCT&HRB.

Housing

Animals will be kept in polycarbonate cages 26×17×12 cm with bedding, 5-6 animals per cage. The cages have steel lattice covers with deepenings, steel dispensers for feed and water, and steel label holders.

Diet

Animals will be fed by conventional granulated feed "Combikorm PK-120 for laboratory rats, mice, and hamsters" (OOO "Laboratorkorm", Russia, 115478, Moscow, Kashirskoye shosse, 24).

Water

Animals will be given water in accordance with GOST "Drinking water" 28.74–73.

Environmental conditions

Laboratory animals will be kept in controlled conditions, temperature 18-20^{0}C, relative humidity 50-60%. Temperature and humidity are monitored in each experimental room and data are recorded by special computer program and once daily in manual datasheets. Light- artificial, 12 h light/12 h dark regime, air exchange – 15/h.

Quarantine: at least 14 days.

7. Study design

Animal grouping

Animals will be assigned to experimental groups at random based on body weight (deviation $\pm$ 20%). In the experiments only young, healthy, sexually mature animals will be taken. Animal species is chosen in compliance with the Methodical guidance (see item 4). Number of animals in group – 5 per sex.

Doses, routes of administration, repeatability

Mutagenic effect is normally studied at a single intraperitoneal administration and at a repeated administration as intended routes for clinical practice. Mutagenic activity of the test substance at a single exposure in two doses: 1/5 LD_{50} and in the dose equivalent to a daily therapeutic dose

for humans (TD) calculated for experimental animals based on body surface area was studied earlier.

In this study mutagenic effect of 0611 substance at 4-repeated peroral administration will be assessed. The substance will be administered during four days, once daily, in doses 1 TD and 10 TD, 5 male and 5 female mice per dose. Based on correlation of the body surface area of mice and humans the doses will be: 1 TD for humans – 40 mg, 1 TD for mice =0.13 mg/animal; 10 TD=1.33 mg/animal.

Positive control: a mutagen - cyclophosphamide - will be administered in a single dose 20 mg/kg intraperitoneally (for mouse with body weight 20 g – 0.40 mg/animal).

Table 1. Animal groups, dose/volume correlation

C_{57}Bl/6 mice		Substance	Dose		Suspension/solutions	Administration	
Sex	#		(TD)	(mg/animal)		Volume (ml)	Route
Male	1-5	0611	1 TD	0.13	C=6.50 мг/мл	0.02	peros
Female	6-10	0611	1 TD	0.13	C=6.50 мг/мл	0.02	peros
Male	11-15	0611	10 TD	1.33	C=66.5 мг/мл	0.02	peros
Female	16-20	0611	10 TD	1.33	C=66.50 мг/мл	0.02	peros
Male	21-25	C^+	–	0.40	C=2.0 мг/мл	0.2	i/p*
Male	26-30	C^-	–	–	–	0.02	peros
Female	31-35	C^-	–	–	–	0.02	peros
Note*: i/p – intraperitoneal route, a single dose							

7.1. Clinical observation

Daily observation of physical state of animals (motor activity, eye mucosa, skin, defecation).

7.2. Completion of dosing, euthanasia

In 24 hours after the dosing/last dose of the test and control substances the experiment will be completed. Upon completion of the experiment mice will be euthanized by CO_2 inhalation.

From euthanized animals thigh-bones will be taken out. Isolation of bone marrow cells and preparation of material for cytogenetic analysis will be conducted according to the effective SOPs. The obtained preparations (two glasses from each animal) will be coded and passed for microscopic cytogenetic analysis.

8. Microscopic analysis

Microscopic analysis of cytogenetic preparations from murine bone marrow will be performed according to appropriate SOP. For the analysis 100 metaphases from each animal will be taken. Rounded metaphase plates without chromosome overlaying (modal number for mice - 40) will be examined. The number of single and pair fragments, chromatid and chromosomal exchanges, achromatic gaps and cuts in centromere, the number of cells with multiple damages of chromosomes and cells with complete destruction of chromosomes will be counted. The percent of damaged cells for each animal and for each experimental group will be calculated. Results will be documented in a table (see Table 2).

Table 2

Summarized table of results of cytogenetic activity assessment in chromosome aberration assay on mammalian bone marrow cells

Conditions of experiment	Mouse #	Number of tested cells	Number of aberrations			Cells with multiple aberrations	Gaps, number/cells (%)	Damaged cells, %
			Single fragments	Pair fragments	Exchanges			

9. Protocol amendments

Changes in the approved protocol will be discussed by the Study Director and Customer and documented in the form of Amendment which will be approved and will have an effective date. The protocol changes are signed by Study Director and Customer Representative and attached to all approved copies of the protocol.

10. Deviations

Any deviations will be documented in the data sheets with assessment of their affect on the study attached to the Final report.

11. Report

Report will be submitted to the Customer upon completion of the study in accordance with all items of the Study Plan and analysis of the obtained material. The study plan, all amendments and deviations will be attached to the report.

12. Statistical analysis of data

For evaluation of experimental data descriptive statistics will be applied: mean values, standard square deviations will be determined and presented in summarized tables. Comparative analysis of data from different groups will be performed. Confidence probability – 95%.

13. Documentation and archive

Study protocol, amendments and deviations, raw data and reporting materials will be stored in the RCT&HRB archive for 2 years.

14. Protocol approval

Customer representative:

_____	_____	_____
Name	**Signature**	**Date**

Study Director:

_____	_____	_____
Name	**Signature**	**Date**

Director of SFES RCT&HRB:

_____	_____	_____
Name	**Signature**	**Date**