TEST REPORT

Acute Oral and Intraperitoneal Toxicity of BIOTECTON®

based on

Acute Toxic Class Method (OECD 423) in Mouse

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Content

1. Outline of study

It was performed an *in vivo* acute toxicity study in 18 mouse, based on the OECD 423 guideline; **„GUIDELINE FOR TESTING OF CHEMICALS",** using oral or intraperitoneal administration. The study was conducted in three dose groups – 3 mice in each – for both oral and intraperitoneal administration, respectively. Animals received 300, 2000 and 5000 mg test substance „BIOTECTON®" for one kilogram of bodyweight as a single oral or intraperitoneal dose. Following two weeks post-treatment period gross pathology and histopathology tests were performed.

2. Test substance

Physical nature, purity, and physico-chemical properties

The test preparation „BIOTECTON®" was clear, transparent, sterile solution. Purity data and description of physico-chemical properties were not available.

Identification data, including CAS number

No data were given.

Vehicle; justification for choice of vehicle

The vehicle of test substance was sterile physiological (0.9% w/v) saline solution. This vehicle is an isotonic and isosmotic solvent, frequently applied in injection liquids as carrier (cf. USP, PhEUR).

3. Test animals

Species/strain used

In this study white laboratory mouse of the BDF1-strain was used.

Microbiological status of the animals

The test animals were bred and kept conventional way, according to their microbiological status.

Number, age and sex of animals

In this test 18 adult, male mice were used. Their age was between 10 and 12 weeks at the start of the trial.

Source, housing conditions, diet

The test animals were originated from National Institute of Oncology (Hungary, Budapest). The mice were kept in standard rodent boxes (40x12.5x12 cm), 3 animals per allocation. The room temperature was between 21-23 °C and the relative air humidity was between 50-65% RH during whole study. The feeding was managed from trough feeders, watering from bottles. Artificial light program was applied with 12 hour light and 12 hour dark periods.
The animals were randomly selected, marked to permit individual identification, and kept in their cages for 7 days prior to dosing to allow for acclimatisation to the laboratory conditions.

4. Test conditions:

Test substance formulation, physical form of the material administered

In this trial sterile watery solution „BIOTECTON®" was administered in laboratory mice (*cf.* section 2. above).

Administration of the test substance, dosing volumes and time of dosing

The animals received a single oral or intraperitoneal dose of the test substance, respectively. The application was performed based on the instructions of the OECD 423 guideline. During the treatments either the diluted (with physiological, sterile saline) or non-diluted (100 and 200 mg/ml) formulation of „BIOTECTON®" was administered. Next table (table 1.) introduces the detailed conditions of the test substance application.

Table 1.

Single PO or IP treatments of the mice with BIOTECTON® (n=18, adult males)#

Id No	Route	Dose (mg/kg bw.)	Body weight prior the application (g)	Applied volume (ml)
1	oral (PO)	300 (0.1 ml/10 g, 30 mg/ml)	40	0.4*
2			35	0.35*
3			37	0.37*
4		2000 (0.2 ml/10 g, 100 mg/ml)	44	0.88
5			35	0.7
6			37	0.75
7		5000 (0.25 ml/10 g, 200 mg/ml)	46	1.15
8			40	1
9			43	1.1
10	intraperitoneal (IP)	300 (0.1 ml/10 g, 30 mg/ml)	35	0.35*
11			38	0.38*
12			38	0.38*
12		2000 (0.2 ml/10 g, 100 mg/ml)	37	0.74
14			41.5	0.83
15			41.5	0.83
16		5000 (0.25 ml/10 g, 200 mg/ml)	41	1
17			45	1.1
18			44	1.1

#*The data are available in original data record sheet as well (cf.* Appendix 1.*).*

* *Diluted sterile solution was applied in these animals (final concentration: 30 mg/ml)*

The test substance in PO group was administered in a single dose by gavage using a stomach tube, and in IP group in a single dose using sterile disposable plastic syringe and needle (G25).

Three animals per route of application were used for each step. First the selected two times three (per oral and intraperitoneal) animals received 300 mg/kg bw. of the test substance BIOTECTON®. Hence, no any animal has died in four hours the test was furthered with the administration of 2000 mg/kg in next 3 plus 3 animals, respectively. Because of the absence of lethal effect in 2000 mg/kg groups another 3 plus 3 mice were treated orally or intraperitoneally with 5000 mg/kg dose after another four hours observation.

Details of food and water quality (including diet type/source, water source)

The test mice received standard rodent feed and water in drinking quality *ad libitum* during the whole trial. On day of the administration of test product the animals were fasted, feeding was withheld for 4 hours prior the application. Mice received first feed 2 hours thereafter the drug administration.

The rationale for the selection of the starting dose

The active ingredients of „BIOTECTON®" are with plant origin (herbal remedy). Similar active substances can be considered as practically non toxic substances. However, there was no information on a substance to be tested. For animal welfare reasons it was recommended to use the starting dose of 300 mg/kg body weight (*cf.* OECD 423, Annex 2 c: Starting dose is: 300 mg/kg bw.)

5. Results

Tabulation of response data and dose level for each animal

Animals were observed individually after dosing once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days. The following table (Table 2.) introduces the results of

clinical observation. Observations included changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behaviour pattern.

Table 2.

Individual records on clinical obsevations

Id No	Route	Dose (mg/kg bw.)	First day (hours)				Post-treatment period (days)													
			0.5	4	8	12	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	PO	300 (0.1 ml/10 g, 30 mg/ml)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2			N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3			N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
4		2000 (0.2 ml/10 g, 100 mg/ml)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5			N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
6			N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
7		5000 (0.25 ml/10 g, 200 mg/ml)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
8			N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
9			N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
10	IP	300 (0.1 ml/10 g, 30 mg/ml)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
11			N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
12			N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
12		2000 (0.2 ml/10 g, 100 mg/ml)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
14			N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
15			N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
16		5000 (0.25 ml/10 g, 200 mg/ml)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
17			N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
18			N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

N= negative

Evidence of any clinical sign of toxic reaction was detected neither in lower nor in high doses according to the PO or IP treated groups of mice.

Tabulation of body weight and body weight changes

Individual weights of animals at the day of dosing, in weekly intervals thereafter, and at time of death or sacrifice are introduced in table 3.

Table 3.

Individual weights of animals

Id No	Route	Dose (mg/kg bw.)	Body weight (g)		
			Before treatment	Day 7	Sacrifice
1	PO	300 (0.1 ml/10 g, 30 mg/ml)	40	42.5	43.3
2			35	35.8	36.7
3			37	37	36.0
4		2000 (0.2 ml/10 g, 100 mg/ml)	44	45	45.3
5			35	36.6	38.3
6			37	36	35.5
7		5000 (0.25 ml/10 g, 200 mg/ml)	46	46.7	47.6
8			40	40	41.0
9			43	44	45.0
10	IP	300 (0.1 ml/10 g, 30 mg/ml)	35	35	35.2
11			38	39	40.6
12			38	38	38.8
12		2000 (0.2 ml/10 g, 100 mg/ml)	37	38	38.5
14			41.5	42.3	43.9
15			41.5	42	42.8
16		5000 (0.25 ml/10 g, 200 mg/ml)	41	41.8	42.4
17			45	47	48.2
18			44	45	46.3

Date and time of death if prior to scheduled sacrifice

Prior the scheduled sacrifice not a single animal has died.

Time course of onset of signs of toxicity

In the time course of post treatment period no sign of toxic reaction was observed. Each animal remained healthy and survived the two weeks of the post-treatment observation period.

Necropsy and histopathological findings

All test animals participated in this trial survived, and at the end of 2 weeks observation period they were subjected to gross necropsy. Animals were euthanized with the help of cervical dislocation. Findings of gross pathology examination and the weights of the liver, spleen, and right kidney were recorded individually (*cf.* Appendix 2.). The results of necropsy and the weights of the selected organs are introduced in table 4.

Furthermore, liver, spleen, kidney, lung, heart, and small intestine tissue samples were sent to histopathology examination. The results of this test as well as the photos on histopathology examinations are introduced in Appendix 3.

In 11 mice of 18 fatty infiltration of the liver was detected, as the consequence of good body condition.

The minor alterations in internal organs detected with histopathology are vital reactions, and can not be considered as the consequence of supposed toxic effect of test substance. In six animals (No 1, 4, 7, 12, 16, 18) the liver was hyperaemic, and the lung tissue was hyperaemic only in animal No 7. In spleen tissue lymphoid depletion was observed in animals 6 and 7. In the same tissue the signs of *megacytosis* were detected in one PO and three IP treated animals. However, these alterations do not appear to be in strong positive correlation with the applied dose. In animal No 5 slight segmental tubular nephrosis was seen (possibly due to other harming effect). The fusion of the intestinal villi occurred in 8 animals of 18, possibly not as the result of the administration of test substance.

Table 4.

Necropsy findings, and weights of internal organs

Id No	Route	Dose (mg/kg bw.)	Weight (g) of			Sacrifice findings
			liver	r. kidney	spleen	
1	PO	300 (0.1 ml/10 g, 30 mg/ml)	1.98	0.35	0.22	Intact peritoneum and inter. organs
2			1.79	0.27	0.13	Intact peritoneum and inter. organs
3			1.99	0.27	0.11	Intact peritoneum and inter. organs
4		2000 (0.2 ml/10 g, 100 mg/ml)	2.54	0.39	0.11	Intact peritoneum and inter. organs, mild obesity
5			2.29	0.33	0.13	Intact peritoneum and inter. organs
6			1.85	0.27	0.14	Intact peritoneum and inter. organs
7		5000 (0.25 ml/10 g, 200 mg/ml)	2.68	0.37	0.14	Intact peritoneum and inter. organs, obesity
8			2.37	0.28	0.11	Intact peritoneum and inter. organs
9			2.53	0.38	0.22	Intact peritoneum and inter. organs, week splenomegaly
10	IP	300 (0.1 ml/10 g, 30 mg/ml)	1.58	0.27	0.12	Intact peritoneum and inter. organs
11			2.25	0.31	0.14	Intact peritoneum and inter. organs
12			2.05	0.47	0.15	Intact peritoneum and inter. organs
12		2000 (0.2 ml/10 g, 100 mg/ml)	2.20	0.37	0.16	Intact peritoneum and inter. organs
14			2.46	0.34	0.19	Intact peritoneum and inter. organs
15			2.41	0.38	0.20	Intact peritoneum and inter. organs
16		5000 (0.25 ml/10 g, 200 mg/ml)	2.18	0.44	0.18	Intact peritoneum and inter. organs
17			2.48	0.44	0.17	Intact peritoneum and inter. organs, slightly failed spleen development
18			2.94	0.43	0.22	Intact peritoneum and inter. organs

6. Discussion and interpretation of results

Acute oral toxicity refers to those adverse effects occurring following oral administration of a single dose of a substance, or multiple doses given within 24 hours. Delayed death means that an animal does not die or appear moribund within 48 hours but dies later during the 14-day observation period (OECD 423). According to the administration of BIOTECTON® in 18 healthy, young-adult, male mice neither adverse effects nor delayed death occurred. Although, during the histopathology examination slight signs of splenotoxicity (i.e. megacytosis) and some other minor alteration of internal organs were observed in several treated animals, the absence of clinical signs and lethality indicates the high safety of BIOTECTON®.

7. Conclusions

In this study, conducted on 18 healthy, young-adult, male mice, the test substance BIOTECTON® can be considered as practically non toxic agent according to both *per os* (PO) and intraperitoneal (IP) application. After the single application of 5000 mg/kg BIOTECTON®, as the upper limitation on testing (OECD 423), neither clinical sign of toxicity nor lethal effect could be detected. BIOTECTON® can be considered as practically harmless substance, regarding to the Globally Harmonised Classification System for Chemical Substances (GHS).

8. Appendices

Appendix 1.

Individual data records on treatments

Appendix 2.

Individual data records on sacrifice

Appendix 3.

Expert report on histopathology examination

BUDAPEST, 18-02-2014

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György Csikó, DVM, CSc. Dr. Habil	János Gál, DVM, PhD. Dr. Habil
Associate professor	Associate professor
Study director	Pathologist

BIOTECTON®
Egér akut toxicitás

Kezelés 2014. 01. 17. 10-11

Beadás	Dózis mg/ttkg	No	Testtömeg (g)	Beadott ml	Megfigyelés
PO	300& (zöld)	1	40	0,4	
		2	35	0,35	
		3	37	0,37	
	2000# (kék)	4	44	0,88	
		5	35	0,7	
		6	37	0,75	
	5000$ (piros)	7	46	1,15	
		8	40	1	
		9	43	1,1	
IP	300& (zöld)	10	35	0,35	
		11	38	0,38	
		12	38	0,38	
	2000# (kék)	13	37	0,74	
		14	41,5	0,83	
		15	41,5	0,83	
	5000$ (piros)	16*	41	1	vérzik
		17	45	1,1	
		18	44	1,1	

* 16B (jel változás) – 47 g 1,2 ml vérzés

DÓZISOK (koncentráció-térfogat összefüggés):

& 300 mg/ttkg: konc. 30 mg/ml, adag 0,1 ml/10 g (100 mg/ml-ből hígítani – 3,33 x-ra)

2000 mg/ttkg: konc. 100 mg/ml, adag 0,2 ml/10 g

$ 5000 mg/ttkg: konc. 200 mg/ml, adag 0,25 ml/10g

BONCOLÁSI JEGYZŐKÖNYV
BIOTECTON AKUT TOXICITÁSI VIZSGÁLAT
2014.01.31.

Adagolás	No	Szervtömeg (g)	Megfigyelés
P.O. 300 mg/ttkg	1	Máj: 1,38 *Vesék: 0,35 Lép: 0,22	43,3 g ♀
	2	Máj: 1,79 Vesék: 0,27 Lép: 0,129	36,7 g ♀
	3	Máj: 1,99 Vesék: 0,27 Lép: 0,105	36,04 g ♀
P.O. 2000 mg/ttkg	4	Máj: 2,54 Vesék: 0,39 Lép: 0,107	45,33 g [illegible]
	5	Máj: 2,29 Vesék: 0,33 Lép: 0,133	38,28 g
	6	Máj: 1,85 Vesék: 0,27 Lép: 0,140	35,5 g
P.O. 5000 mg/ttkg	7	Máj: 2,675 Vesék: 0,37 Lép: 0,140	47,6 g [illegible]
	8	Máj: 2,370 Vesék: 0,28 Lép: 0,111	40,96 g
	9	Máj: 2,53 Vesék: 0,38 Lép: 0,223	45,0 g [illegible] [illegible]

* csak jobb vese

BONCOLÁSI JEGYZŐKÖNYV
BIOTECTON AKUT TOXICITÁSI VIZSGÁLAT

2014.01.31.

Adagolás	No	Szervtömeg (g)	Megfigyelés
IP. 300 mg/ttkg	10	Máj: 1,58 g Vesék: 0,27 g Lép: 0,115 g	35,24 g hashártya ép, kóros elváltozásoktól mentes
	2 11	Máj: 2,25 Vesék: 0,31 Lép: 0,14	40,64 g hashártya ép
	3 12	Máj: 2,05 Vesék: 0,47 Lép: 0,15	38,75 g hashártya ép
IP. 2000 mg/ttkg	4 13	Máj: 2,2 Vesék: 0,365 Lép: 0,16	38,46 g hashártya ép
	5 14	Máj: 2,46 Vesék: 0,34 Lép: 0,187	43,9 g hashártya ép
	6 15	Máj: 2,41 Vesék: 0,380 Lép: 0,198	42,75 g hashártya ép
IP. 5000 mg/ttkg	7 16	Máj: 2,175 Vesék: 0,435 Lép: 0,176	42,38 g hashártya ép
	8 17	Máj: 2,48 Vesék: 0,44 Lép: 0,165	48,225 g hashártya ép lép fejlődési hiba, melléklépe van
	9 18	Máj: 2,937 Vesék: 0,428 Lép: 0,214	46,27 g hashártya ép

Szakvélemény

Készítette:

Dr. Gál János PhD., Dipl. ECZM

2014

Dr. Sichtnik László kérésére, 2014. január 31.-én Dr. Csikó György által a SzIE-ÁOTK, Gyógyszertani és Méregtani Tanszékének állatházában vizsgálatba vont, 18 kifejlett, konvencionális státuszú, hím egér diagnosztikai boncolását követően Dr. Gál János SzIE-ÁOTK, Egzotikus Állat- és Vadegészségügyi Tanszékén kórszövettani vizsgálatot végzett.

A vizsgálatba vont egerek, hatályos állatvédelmi szabályok megtartása mellett elvégzett eutanáziáját követően boncolásra kerültek. A boncolás után a lépből, a májból, a veséből, a vékonybélből, a szívből és a tüdőből kis darabkák 8%-os formaldehid oldatban fixálódtak. A rögzítést és paraffinba ágyazást követően 3-4 µm-es metszetek kerültek metszésre, majd tárgylemezre rögzítést követően hematoxilin-eozin festésre. A fedésüket követően mikroszkópos vizsgálat történt. A vizsgálat eredményét az 1. táblázat tartalmazza.

Sorszám	Szerv					
	Máj	Lép	Vese	Tüdő	Szív	Vékonybél
1	rendes, bővérűség jelei	rendes	rendes	rendes	a rostok inhomogén festődése, a szívburok részleges megvastagodása	rendes
2	rendes, kisfokú zsíros elfajulás	rendes	rendes	rendes	rendes	a bélbolyhok fúziójával kísért boholy atrophia
3	rendes, kisfokú zsíros elfajulás	rendes	rendes	rendes	rendes	rendes
4	rendes, kisfokú zsíros elfajulás és bővérűség	rendes	rendes	rendes	rendes	rendes
5	rendes, kisfokú zsíros elfajulás	rendes	diszkrét segmentalis tubulonephrosis	rendes	rendes	a bélbolyhok részleges fúziója
6	rendes, kisfokú zsíros elfajulás	kisfokú lymphoid depléció	a metszetkészítési technikai hiba folytán nem vizsgálható	rendes	rendes	a bélbolyhok részleges fúziója
7	rendes, kisfokú zsíros elfajulás és bővérűség	kisfokú lymphoid depléció	rendes	kisfokú bővérűség	a rostok inhomogén festődése, a szívburok részleges megvastagodása	rendes
8	multifokális zsíros infiltráció	rendes	mérsékelt tubulonefrózis	rendes	a rostok inhomogén festődése, mérsékelt perivasculitis	a bélbolyhok fúziója
9	multifokális zsíros infiltráció	közepes fokú megacariocytosis	rendes	rendes	a rostok inhomogén festődése	rendes
10	rendes	rendes	rendes	rendes	rendes	rendes
11	multifokális zsíros infiltráció	a metszetkészítési technikai hiba folytán nem vizsgálható	rendes	rendes	rendes	rendes
12	bővérűség	rendes	rendes	rendes	a rostok helyenként feltöredeztek	rendes
13	kisfokú zsíros elfajulás	rendes	rendes	rendes	inhomogén festődés	bélbolyhok fúziója
14	rendes	közepes fokú megacariocytosis	rendes	rendes	rendes	bélbolyhok fúziója
15	kisfokú zsíros elfajulás	közepes fokú megacariocytosis	rendes	rendes	rendes	rendes
16	bővérűség	rendes	rendes	rendes	rendes	bélbolyhok fúziója
17	multifokális zsíros infiltráció	enyhe fokú megacariocytosis	rendes	rendes	rendes	bélbolyhok fúziója
18	bővérűség	rendes	rendes	rendes	inhomogén festődés	rendes

A vizsgálatba vont egerek szerveiről készült metszetek fotóit egyedenként csoportosítva mellékletben csatoltuk.

Budapest, 2014. február 11.

Dr. Gál János PhD. Dipl. ECZM