TEST REPORT

Acute Oral and Intraperitoneal Toxicity of BIOTECTON®

based on

Acute Toxic Class Method (OECD 423) in Mouse

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***Editorial and product naming note**
This study was originally conducted in 2014 under the product name BIOTECTON®, which was subsequently renamed VIROPLAZIN®. This document has been reviewed and edited for grammar, spelling, formatting, and readability. The original scientific findings, methodology, data, and conclusions have been preserved without intentional alteration.
The original product name has been retained throughout the report to preserve the integrity of the historical scientific documentation.

Content

1. Outline of study **3**

2. Test substance **3**

Physical nature, purity, and physico-chemical properties 3

Identification data, including CAS number 3

Vehicle; justification for choice of vehicle 3

3. Test animals **4**

Species/strain used 4

Microbiological status of the animals 4

Number, age and sex of animals 4

Source, housing conditions, diet 4

4. Test conditions: **5**

Test substance formulation, physical form of the material administered 5

Administration of the test substance, dosing volumes and time of dosing 5

Details of food and water quality (including diet type/source, water source) 7

The rationale for the selection of the starting dose 7

5. Results **8**

Tabulation of response data and dose level for each animal 8

Tabulation of body weight and body weight changes 10

Time course of onset of signs of toxicity 11

Necropsy and histopathological findings 11

6. Discussion and interpretation of results **14**

7. Conclusions **14**

8. Appendices **15**

1. Outline of study

An *in vivo* acute toxicity study was performed in 18 mice, based on the OECD 423 guideline, **"GUIDELINE FOR TESTING OF CHEMICALS",** using oral and intraperitoneal administration. The study was conducted in three dose groups: 3 mice for both oral and intraperitoneal administration, respectively. Animals received 300, 2000, and 5000 mg of the test substance, "BIOTECTON®" for one kilogram of body weight as a single oral or intraperitoneal dose. Following a two-week 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 a clear, transparent, sterile solution. Purity data and description of physico-chemical properties were not available.

Identification data, including CAS number

No data was given.

Vehicle; justification for choice of vehicle

The vehicle of the 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 a carrier (cf. USP, PhEUR).

3. Test animals

Species/strain used

In this study, white laboratory mice of the BDF1 strain were used.

Microbiological status of the animals

The test animals were bred and kept in a 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 obtained from the 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 the whole study. Feeding was managed from trough feeders, and watering from bottles. An 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 aqueous 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. The 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 to the application (g)	Applied volume (ml)
1	oral (PO)	300 (0.1 ml/10g, 30 mg/ml)	40	0.4*
2			35	0.35*
3			37	0.37*
4		2000 (0.2 ml/10g, 100 mg/ml)	44	0.88
5			35	0.7
6			37	0.75
7		5000	46	1.15

8		**(0.25 ml/10g, 200 mg/ml)**	**40**	**1**
9			**43**	**1.1**
10	**intraperitoneal (IP)**	**300 (0.1 ml/10g, 30 mg/ml)**	**35**	**0.35***
11			**38**	**0.38***
12			**38**	**0.38***
13		**2000 (0.2 ml/10g, 100 mg/ml)**	**37**	**0.74**
14			**41.5**	**0.83**
15			**41.5**	**0.83**
16		**5000 (0.25 ml/10g, 200 mg/ml)**	**41**	**1**
17			**45**	**1.1**
18			**44**	**1.1**

[#]*The data are available in the 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 the PO group was administered in a single dose by gavage using a stomach tube, and in the IP group in a single dose using a 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 animal died within four hours; the test was continued with the administration of 2000 mg/kg in the next 3 plus 3 animals, respectively. Because of the absence of lethal effect in the 2000 mg/kg groups, another 3 plus 3 mice were treated orally or intraperitoneally with a 5000 mg/kg dose after another four hours of observation.

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

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

The rationale for the selection of the starting dose

The active ingredients of "BIOTECTON®" are of 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) presents 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 observations

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/10g, 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/10g, 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/10g, 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/10g, 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**
13		**2000 (0.2 ml/10g, 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/10g, 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

No evidence of any clinical sign of toxic reaction was detected in either the lower or the high doses according to the PO or IP treated groups of mice.

Tabulation of body weight and body weight changes

Individual weights of animals on the day of dosing, at 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/10g, 30 mg/ml)	40	42.5	43.3
2			35	35.8	36.7
3			37	37	36
4		2000 (0.2 ml/10g, 100 mg/ml)	44	45	45.3
5			35	36.6	38.3
6			37	36	35.5
7		5000 (0.25 ml/10g, 200 mg/ml)	46	46.7	47.6
8			40	40	41
9			43	44	45
10	IP	300 (0.1 ml/10g, 30 mg/ml)	35	35	35.2
11			38	39	40.6
12			38	38	38.8
13		2000 (0.2 ml/10g, 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/10g, 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 to the scheduled sacrifice, not a single animal had died.

Time course of onset of signs of toxicity

In the time course of the 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 presented 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 of the histopathology examinations, are introduced in Appendix 3.

In 11 mice of 18 fatty infiltration of the liver was detected, as a 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 the supposed toxic effect of the 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, 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 harmful effects). The fusion of the intestinal villi occurred in 8 animals of 18, possibly not as a result of the administration of the 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/10g, 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/10g, 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/10g, 200 mg/ml)**	**2.68**	**0.37**	**0.14**	Intact peritoneum and inter. organs, mild 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, weak splenomegaly
10	**IP**	**300 (0.1 ml/10g, 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
13		**2000 (0.2 ml/10g, 100 mg/ml)**	**2.2**	**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**	**0.2**	Intact peritoneum and inter. organs
16		**5000 (0.25 ml/10g, 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 a 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 limit for testing (OECD 423), neither clinical signs of toxicity nor lethal effects could be detected. BIOTECTON® can be considered a practically harmless substance, according 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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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	vérzés
		17	45	1,1	
		18	44	1,1	

* 16B (jel valószínű) – 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 JEGYZŐKŐNYV
BIOTECTON AKUT TOXICITÁSI VIZSGÁLAT
2014.01.31.

Adagolás	No	Szervtömeg (g)	Megfigyelés
P.O. 300 mg/ttkg	1	Máj: 1,98 *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]

* Csak jobb vese

BONCOLÁSI JEGYZŐKŐNYV
BIOTECTON AKUT TOXICITÁSI VIZSGÁLAT
2014.01.31.

Adagolás	No	Szervtömeg (g)	Megfigyelés
IP. 300 mg/ttkg	1 0	Máj: 1,58 g Vesék: 0,27 g Lép: 0,115 g	35,24 g hasháztya ép, kóros elváltozásoktól mentes
	2 11	Máj: 2,25 Vesék: 0,31 Lép: 0,14	40,64 g hasháztya ép
	3 12	Máj: 2,05 Vesék: 0,47 Lép: 0,15	38,75 g hasháztya ép
IP. 2000 mg/ttkg	4 13	Máj: 2,2 Vesék: 0,365 Lép: 0,16	38,46 g hasháztya ép
	5 14	Máj: 2,46 Vesék: 0,34 Lép: 0,187	43,9 g hasháztya ép
	6 15	Máj: 2,41 Vesék: 0,380 Lép: 0,198	42,75 g hasháztya ép
IP. 5000 mg/ttkg	7 16	Máj: 2,175 Vesék: 0,435 Lép: 0,176	42,38 g hasháztya ép
	8 17	Máj: 2,48 Vesék: 0,44 Lép: 0,165	48,225 g hasháztya ép lép fejlődési hiba, melléklépe van
	9 18	Máj: 2,937 Vesék: 0,428 Lép: 0,217	46,27 g hasháztya ép