Tables

Table 1.

Lethality

Post-treatment observation period (14 days)

	Group 1	**Group 2**
	MALES	FEMALES
Treatment	death / number of animals	
N-acetyl-L-hydroxyproline; 2,000 mg/kg, po.	0/10	0/10

Table 2.

Clinical Symptoms

Post-treatment observation period (14 days)

	Group 1	**Group 2**
	MALES	FEMALES
Treatment	symptom / number of animals	
N-acetyl-L-hydroxyproline; 2,000 mg/kg, po.	0/10	0/10

Acute oral toxicity study of N-acetyl-L-hydroxyproline with 14-day post-treatment observation period in the rat (limit test)

Table 3.1.

Body Weights

MALES

Group 1 Treatment	Body weights [g]					
	Day of arrival	Day of randomi-zation	Day 1 prior to treatment	Day 2	Day 8	Day 15
N-acetyl-L-hydroxyproline; 2,000 mg/kg, po.						
Group size:	10	10	10	10	10	10
Mean:	151.4	203.4	189.3	200.2	260.3	314.9
± S.D.:	6.33	7.75	7.47	8.18	12.60	18.71

FEMALES

Group 2 Treatment	Body weights [g]					
	Day of arrival	Day of randomi-zation	Day 1 prior to treatment	Day 2	Day 8	Day 15
N-acetyl-L-hydroxyproline; 2,000 mg/kg, po.						
Group size:	10	10	10	10	10	10
Mean:	140.7	165.6	155.4	167.2	191.2	214.3
± S.D.:	0.59	4.10	3.82	5.28	8.89	11.06

Table 3.2.

Body Weight Changes

MALES

Group 1 Treatment	Body weight changes* [g]		
	Day 1 through Day 2	Day 2 through Day 8	Day 8 through Day 15
N-acetyl-L-hydroxyproline; 2,000 mg/kg, po.			
Group size:	10	10	10
Mean:	10.9	60.1	54.6
± S.D.:	3.13	7.54	8.30

FEMALES

Group 2 Treatment	Body weight changes* [g]		
	Day 1 through Day 2	Day 2 through Day 8	Day 8 through Day 15
N-acetyl-L-hydroxyproline; 2,000 mg/kg, po.			
Group size:	10	10	10
Mean:	11.8	24.0	23.1
± S.D.:	3.61	7.43	5.53

* Individual body weight changes were calculated from body weights weighed on Days 1 and 2, Days 2 and 8 as well as Days 8 and 15

Table 4.

Gross Pathology Findings

	Group 1		Group 2	
	MALES		FEMALES	
Treatment	external	internal	external	internal
	finding / number of animals			
N-acetyl-L-hydroxyproline; 2,000 mg/kg, po.	0/10	0/10	0/10	4*/10

* hydrometra

Appendices

Acute oral toxicity study of N-acetyl-L-hydroxyproline with 14-day post-treatment observation period in the rat (limit test)

Appendix 1.1.

Individual Data of Lethality

MALES

Group 1 Animal code	DAYS OF OBSERVATION PERIOD														
	Day 1*	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15
N-acetyl-L-hydroxyproline; 2,000 mg/kg, po.															
21	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
22	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
23	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
24	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
25	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
26	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
27	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
28	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
29	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
30	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ

Remarks: θ = No Lethality

* Day 1 = Treatment's day

Acute oral toxicity study of N-acetyl-L-hydroxyproline with 14-day post-treatment observation period in the rat (limit test)

Appendix 1.2.

Individual Data of Lethality

FEMALES

Group 2 Animal code	DAYS OF OBSERVATION PERIOD														
	Day 1*	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15
N-acetyl-L-hydroxyproline; 2,000 mg/kg, po.															
31	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
32	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
33	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
34	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
35	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
36	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
37	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
38	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
39	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
40	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ

Remarks: θ = No Lethality

* Day 1 = Treatment's day

Acute oral toxicity study of N-acetyl-L-hydroxyproline with 14-day post-treatment observation period in the rat (limit test)

Appendix 2.1.

Individual Clinical Symptoms

MALES

Group 1 Animal code	DAYS OF OBSERVATION PERIOD														
	Day 1*	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15
N-acetyl-L-hydroxyproline; 2,000 mg/kg, po.															
21	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
22	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
23	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
24	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
25	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
26	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
27	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
28	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
29	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
30	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF

Remarks: SF = Symptom Free

* Day 1 = Treatment's day

Appendix 2.2.

Individual Clinical Symptoms

FEMALES

Group 2 Animal code	DAYS OF OBSERVATION PERIOD														
	Day 1*	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15
N-acetyl-L-hydroxyproline; 2,000 mg/kg, po.															
31	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
32	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
33	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
34	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
35	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
36	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
37	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
38	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
39	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
40	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF

Remarks: SF = Symptom Free

* Day 1 = Treatment's day

Appendix 3.1.

Individual Body Weights

MALES

Group 1	Body weights [g]					
Animal code	Day of arrival	Day of randomi-zation	Day 1 prior to treatment	Day 2	Day 8	Day 15
N-acetyl-L-hydroxyproline; 2,000 mg/kg, po.						
21	161.3	212.8	195.5	209.0	274.4	328.1
22	158.3	208.5	197.2	205.2	260.2	315.4
23	152.2	205.8	194.3	200.1	268.9	332.6
24	149.6	200.3	186.0	193.1	255.6	315.5
25	140.4	190.0	174.8	185.5	248.1	307.4
26	155.3	210.8	195.5	208.9	269.8	326.7
27	153.4	207.5	193.6	205.6	261.0	304.4
28	152.1	205.1	187.2	202.1	267.0	323.5
29	146.5	202.5	189.4	203.1	265.9	326.7
30	144.6	190.9	179.6	189.4	231.7	268.6
Group size:	10	10	10	10	10	10
Mean:	**151.4**	**203.4**	**189.3**	**200.2**	**260.3**	**314.9**
± S.D.:	6.33	7.75	7.47	8.18	12.60	18.71

Appendix 3.2.

Individual Body Weights

FEMALES

Group 2	Body weights [g]					
Animal code	Day of arrival	Day of randomi-zation	Day 1 prior to treatment	Day 2	Day 8	Day 15
N-acetyl-L-hydroxyproline; 2,000 mg/kg, po.						
31	141.8	170.7	161.2	173.5	204.8	228.0
32	140.6	168.3	155.4	167.4	195.6	216.3
33	140.7	164.9	152.7	166.2	190.8	216.2
34	141.6	162.2	153.0	156.8	187.7	210.0
35	140.3	160.0	150.1	163.1	182.0	197.7
36	140.5	170.5	159.7	175.6	192.2	211.2
37	140.5	169.2	159.0	168.9	198.1	215.9
38	140.9	167.2	157.9	167.1	196.7	232.0
39	140.1	162.6	152.6	169.0	190.9	217.5
40	140.0	160.5	152.1	164.3	173.0	197.7
Group size:	10	10	10	10	10	10
Mean:	**140.7**	**165.6**	**155.4**	**167.2**	**191.2**	**214.3**
± S.D.:	0.59	4.10	3.82	5.28	8.89	11.06

Appendix 3.3.

Individual Body Weight Changes

MALES

Group 1	Body weight changes* [g]		
Animal code	Day 1 through Day 2	Day 2 through Day 8	Day 8 through Day 15
N-acetyl-L-hydroxyproline; 2,000 mg/kg, po.			
21	13.5	65.4	53.7
22	8.0	55.0	55.2
23	5.8	68.8	63.7
24	7.1	62.5	59.9
25	10.7	62.6	59.3
26	13.4	60.9	56.9
27	12.0	55.4	43.4
28	14.9	64.9	56.5
29	13.7	62.8	60.8
30	9.8	42.3	36.9
Group size:	10	10	10
Mean:	**10.9**	**60.1**	**54.6**
± S.D.:	3.13	7.54	8.30

* Individual body weight changes were calculated from body weights weighed on Days 1 and 2, Days 2 and 8 as well as Days 8 and 15

Appendix 3.4.

Individual Body Weight Changes

FEMALES

Group 2	Body weight changes* [g]		
Animal code	Day 1 through Day 2	Day 2 through Day 8	Day 8 through Day 15
N-acetyl-L-hydroxyproline; 2,000 mg/kg, po.			
31	12.3	31.3	23.2
32	12.0	28.2	20.7
33	13.5	24.6	25.4
34	3.8	30.9	22.3
35	13.0	18.9	15.7
36	15.9	16.6	19.0
37	9.9	29.2	17.8
38	9.2	29.6	35.3
39	16.4	21.9	26.6
40	12.2	8.7	24.7
Group size:	10	10	10
Mean:	**11.8**	**24.0**	**23.1**
± S.D.:	3.61	7.43	5.53

* Individual body weight changes were calculated from body weights weighed on Days 1 and 2, Days 2 and 8 as well as Days 8 and 15

Appendix 4.1.

Gross Pathology Findings

MALES

Group 1 Animal code	Day 15	
	external	internal
N-acetyl-L-hydroxyproline; 2,000 mg/kg, po.		
21	No Finding	No Finding
22	No Finding	No Finding
23	No Finding	No Finding
24	No Finding	No Finding
25	No Finding	No Finding
26	No Finding	No Finding
27	No Finding	No Finding
28	No Finding	No Finding
29	No Finding	No Finding
30	No Finding	No Finding

„No Finding" stays here for the following text:

external: Animal of average development. Skin, fur, visible mucous membranes are intact.

internal: Organs are without pathological changes.

Gastric mucosa: intact

Appendix 4.2.

Gross Pathology Findings

FEMALES

Group 2 Animal code	Day 15	
	external	internal
N-acetyl-L-hydroxyproline; 2,000 mg/kg, po.		
31	No Finding	*
32	No Finding	No Finding
33	No Finding	No Finding
34	No Finding	No Finding
35	No Finding	No Finding
36	No Finding	**
37	No Finding	No Finding
38	No Finding	No Finding
39	No Finding	*
40	No Finding	**

„No Finding" stays here for the following text:

external: Animal of average development. Skin, fur, visible mucous membranes are intact.

internal: Organs are without pathological changes.

Gastric mucosa: intact

* Uterine horns slightly distended, diam of 3 mm, filled with clear fluid (hydrometra). Gastric mucosa: intact.

** see * plus uterine horns are slightly hyperemic.

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KYOWA

CERTIFICATE OF ANALYSIS

Commodity : N-ACETYL-L-HYDROXYPROLINE
Lot No. : 0145003
Quantity : 1.2 KG
Analysis date : FEB. 20. 2003

SPECIFICATION	ACCEPTANCE CRITERIA	RESULT
APPEARANCE	WHITE CRYSTALLINE POWDER	WHITE CRYSTALLINE POWDER
IDENTIFICATION	PASS TEST	PASS TEST
STATE OF SOLUTION(T%)	NOT LESS THAN 95.0 %	NOT LESS THAN 95.0 %
SPECIFIC ROTATION(AT 20℃)	-127 to -115 °	-119 °
AMMONIUM(NH4)	NOT MORE THAN 0.020 %	NOT MORE THAN 0.020 %
CHLORIDE(Cl)	NOT MORE THAN 0.020 %	NOT MORE THAN 0.020 %
SULFATE(SO4)	NOT MORE THAN 0.028 %	NOT MORE THAN 0.028 %
IRON(Fe)	NOT MORE THAN 10 ppm	NOT MORE THAN 10 ppm
HEAVY METALS(Pb)	NOT MORE THAN 10 ppm	NOT MORE THAN 10 ppm
ARSENIC(As2O3)	NOT MORE THAN 1 ppm	NOT MORE THAN 1 ppm
FOREIGN AMINO ACIDS	NOT DETECTED (TLC 10 μg)	NOT DETECTED (TLC 10 μg)
LOSS ON DRYING	NOT MORE THAN 1.0 %	0.7 %
RESIDUE ON IGNITION	NOT MORE THAN 0.10 %	0.01 %
ASSAY(DRY BASIS)	99.0 to 101.0 %	99.9 %

The undersigned affirms that contents mentioned above are truly reported in accordance with the analysis by Quality Control Center.

M. Hayashi

Bio-Chemicals Company

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HUNGARY

Budapest, 23rd May 2002
No.: 5165/48/2002
Our ref.: Dr Éva Iván/KK
Annex:
Subject:

STATEMENT OF GLP COMPLIANCE

Date of inspection: 16-17 April, 2002

Assessment of conformity with GLP Principles has been performed according to Council Directives 87/18/EEC and 88/320/EEC and Commission Directives 1999/11/EC and 1999/12/EC.

According to the criteria specified in paragraph 5 of Article 3 of the Joint Decree No 9/2001. (III.30.) EüM-FVM of the Minister of Health and the Minister of Agriculture and Regional Development on the application and verification of good laboratory practice the Director-General of the National Institute of Pharmacy certifies that the test facility

Pharmaceutical Control and Development Laboratory Co. Ltd. (PC & DL)
H-1149 Budapest, Mexikói út 9., Hungary

is able to carry out ***toxicity studies and safety pharmacology testing*** in compliance with the Principles of Good Laboratory Practice as established by the OECD and the European Community.

ORSZÁGOS GYÓGYSZERÉSZETI INTÉZET

Prof Sándor Kerpel-Fronius MD., DSc.
Director-General