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FINAL REPORT

Acute Eye Irritation / Corrosion Test of *"Koleston 2000 mit 3 % Lehmannblausulfat"* in Rabbits

Project no.: 10-03-0392-90

Koleston 2000 mit 3% Lehmannblausulfat

12.00%	Destillat Olein
25.00%	Nonylphenolether
2.00%	Laurylethersulfat 28% verd.
3.00%	Glycerin 86%ig
5.00%	Glykoldistearat 90
7.80%	Isopropylalkohol
2.90%	Ammoniak 25%ig
0.49%	Parfüm EDC verte
0.30%	Rexat
0.30%	Ascorbinsäure DAB8
0.10%	Na_2SO_3 wasserfrei
3.20%	Haarbraun
3.00%	Lehmannblausulfat
1.00%	Haargrau
3.90%	Ammoniak 25%ig
5.50%	Ammoniak 25%ig
24.50%	Wasser vollentsalzt

14. SEP. 1990

CONTENTS

	DECLARATION	3
	QUALITY ASSURANCE STATEMENT	4
I.	AIM OF THE TEST	5
II.	SUMMARY	5
III.	MATERIAL AND METHOD	6
IV.	RESULTS	11
V.	GENERAL INFORMATION	13
	APPENDIX I - Diet and Diet Composition	14
	APPENDIX II - Water	15

DECLARATION

We, the undersigned, hereby declare that the work was performed under our supervision and in accordance with the described procedures. We assure that the reported results faithfully reproduce the raw data obtained during the experimental work. To the best of our knowledge, no circumstances have been left unreported which may have affected the quality or integrity of the data or which might have a potential bearing on the validity and reproducibility of this study.

Dr. med. vet. J. Lindena

Scientific supervisor

K. Kaufmann

Study director

17.9.90

Date

QUALITY ASSURANCE STATEMENT

The testing facilities utilized in this study have been inspected regularly in accordance with the principles of Good Laboratory Practice for the testing of chemicals as specified by national (BGB1. I, no. 13, § 19 a, March 22, 1990) and international (OECD, Paris, 1982) legislation.

Procedures relevant to this study were inspected periodically. Inspections were conducted according to the standard operating procedures of the testing facility's quality assurance unit. The final report was audited in detail against the approved protocol and all pertinent raw data. The findings of inspections and audiths were reported to management and to the study director.

B Kunert

(p.p. Quality Assurance Unit)

10.09.90

Date

I. AIM OF THE TEST

The aim of the test was to assess the irritant and/or corrosive effect(s) of **"Koleston 2000 mit 3 % Lehmannblausulfat"** on the eyes and associated mucous membranes. Information derived from this test serves to indicate the existence of possible hazards likely to arise from exposure of the eyes to the test article.

The test was conducted according to the OECD guideline for the testing of chemicals (OECD 405, February 24, 1987). The principles of Good Laboratory Practice for the testing of chemicals as specified by national (BGB1. I, no 13, § 19 a, March 22, 1990) and international (OECD, Paris, 1982) legislation were followed during the performance of the study; the IBR standard study protocol, approved and signed on March 5 and 8, 1990, and the corresponding addendum, signed on June 21, 1990, were valid.

II. SUMMARY

The potential toxicity of **"Koleston 2000 mit 3 % Lehmannblausulfat"** was assessed in an acute eye irritation/corrosion test on 3 albino rabbits. In each animal, 0.1 ml of the test substance was introduced into the conjunctival sac of one eye, the untreated eye serving as a control. Both eyes were examined at 1, 24, 48 and 72 h post applicationem.

Classification

According to the EEC directive 83/467/EEC from July 29, 1983 and the Gefahrstoffverordnung (GefStoffV), 1987 (BGBl. I. p. 2721), the test article

"Koleston 2000 mit 3 % Lehmannblausulfat"

is classified as **"not irritant"**.

III. MATERIAL AND METHOD

1 Animals

Species:	rabbit
Strain:	White New Zealands
Source:	Harald Schriever, Kaninchenfarm, 2740 Bremervörde, Neuendamm 88
Date of receipt:	July 17, 1990
Acclimation period:	at least 7 days
Animal selection:	random
Animal identification:	with individual earmarks; cage labelled with the following information: earmark, sex, date of study initiation, IBR project no.
Weight range at study initiation:	2.4 - 2.8 kg

2 Husbandry

Housing:	individual housing (50 x 45 x 40 cm, L x B x H) in a battery of cages, each equipped with a paper roll disposal system
Illumination:	artificial lighting (120 lux) from 7.00 a.m. - 7.00 p.m.
Temperature:	18 ± 2° C
Relative humidity:	50 - 85 %
Measurement:	with thermohygrometer twice daily

3 Test Article

Name:	Koleston 2000 mit 3 % Lehmannblausulfat
Supplied by:	
Chemical name:	1-Methoxy-2-amino-4-(2`-hydroxyethyl)-amino -benzol-sulfat
Physical state:	grey powder
Batch no.:	not specified by sponsor
Identification:	labelled, where appropriate, with name of test article, batch no., name of sponsor, IBR project no., date of receipt, storage conditions, handling precautions and expiry date
Storage:	ambient, in the dark
Stability:	not specified by sponsor
Solvent/vehicle:	--

4. Test Conditions

Preparation of the animals

Prior to test initiation, all animals were acclimated to laboratory conditions for at least 7 days. 24 h before treatment, the eyes of all animals were examined for potential eye lesions with an ophthalmoscope and healthy animals were subsequently earmarked for individual identification.

Preparation of the test article

The test article was administered undiluted.

Procedure

In each animal, 0.1 ml [not more than 100 mg] of the test article was introduced into the conjunctival sac of the left eye, while the right eye was used as a control.

Observations

Using an ophthalmoscope, ocular reactions were assessed 1, 24, 48 and 72 h after treatment. Extended observations may be necessary to determine reversibility or irreversibility of the lesions observed. On each occasion, ocular irritation and/or corrosion were scored according to the following scheme on the next two pages.

Classification

Eye irritation/corrosion was graded according to the EEC directive 83/467/EEC from July 29, 1983 and to GefStoffV, 1987 (BGBl. I. p. 2721).

GRADING OF OCULAR LESIONS

(as stipulated by OECD 405)

Cornea	Points
Opacity: degree of density (area most dense taken for reading)	
No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal lustre), details of iris clearly visible	1
Easily discernible translucent area, details of iris slightly obscured	2
Nacreous area, no details of iris visible, size of pupil barely discernible	3
Opaque cornea, iris not discernible through the opacity	4

Iris	Points
Normal	0
Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia or injection, any of these or combination of any thereof, iris still reacting to light (sluggish reaction is positive)	1
No reaction to light, haemorrhage, gross destruction (any or all of these)	2

Conjunctivae	Points
Redness (refers to palpebral and bulbar conjunctivae, cornea and iris)	
Blood vessels normal	0
Some blood vessels definitely hyperaemic (injected)	1
Diffuse, crimson colour, individual vessels not easily discernible	2
Diffuse, beefy red	3
Chemosis: lids and/or nictitating membranes	
No swelling	0
Any swelling above normal (including nictitating membranes)	1
Obvious swelling with partial eversion of lids	2
Swelling with lids about half closed	3
Swelling with lids more than half closed	4

IV. RESULTS

Under the experimental conditions described above, the following results were obtained:

Table 1: Individual values of ocular gradings

Animal number	Ocular lesion	Hours after treatment 1	24	48	72
1	Cornea	0	0	0	0
	Iris	0	0	0	0
	Conjunctivae redness	1	1	1	0
	Conjunctivae chemosis	0	0	0	0
2	Cornea	0	0	0	0
	Iris	0	0	0	0
	Conjunctivae redness	1	1	0	0
	Conjunctivae chemosis	0	0	0	0
3	Cornea	0	0	0	0
	Iris	0	0	0	0
	Conjunctivae redness	1	1	1	0
	Conjunctivae chemosis	2	1	0	0

Table 2: Mean values of ocular gradings over the time point is 24, 48 and 72 h

Animal number	Cornea	Iris	Conjunctivae redness	Conjunctivae chemosis
1	.00	.00	.75	.00
2	.00	.00	.50	.00
3	.00	.00	.75	.75

Reversibility

The observed findings were reversible within 3 days p.a.

Classification

According to the EEC directive 83/467/EEC from July 29, 1983 and to GefStoffV, 1987 (BGBl. I. p. 2721), the test article

"Koleston 2000 mit 3 % Lehmannblausulfat"

is classified as **"not irritant"**.

V. GENERAL INFORMATION

Study sponsor:	
Testing facility:	IBR Forschungs GmbH Südkampen Nr. 31 3030 Walsrode 1.
Scientific supervisor:	Priv. Doz. Dr. J. Lindena (Veterinarian, Clinical Biochemist).
Study director:	K. Kaufmann (Veterinarian).
Responsible technicians:	M. Händschke, H. Queren, J. Becker, T. Wiedemann.
Data processing and operations:	Dr. J. Gebert, H. Nünninghoff.
Quality assurance unit:	Dr. A. Burt (Biologist), M. Schaardt (Technician), Dr. B. Kuszewski (Veterinarian), S. Husmann-Holloway (Biochemist), J. Worthmann (Technician).
Experimental period:	July 29 - August 2, 1990.
Archives:	All raw data, documentation and specimens, the protokol, and a copy of the final report will be stored in the IBR archives for the minimum period of time specified by current national and international legislation on GLP. Biological specimens will be retain only as long as they afford a meaningful re-evaluation. At the end of the study, any remaining substance will be returned to the sponsor. IBR reseves the right to retain a sample of the test article.

APPENDIX I - Diet and Diet Composition

Administration:	ad libitum
Manufacturer:	Ssniff Spezialdiäten GmbH 4770 Soest/Westfalen
Name:	Ssniff MÜ Z^R (Alleindiät für Zuchtkaninchen)
Form:	pellets, 1.0-1.5 cm long, 0.5 cm diameter

Composition (per kg diet):

Crude protein	160 g
Crude fat	25 g
Crude fiber	170 g
Crude ash	100 g

Amino acids (per kg diet):

Lysine	8 g
Methionine	2.5 g
Cystine	2 g
Leucine	13.5 g
Isoleucine	9 g
Arginine	9 g
Phenylalanine	8 g
Tryptophan	1.8 g
Histidine	4.5 g
Tyrosine	5.2 g
Valine	8.5 g

Vitamins (supplemented per kg diet):

A	13000 IU
D_3	1300 IU
E	39 mg
B_1	8 mg
B_2	19 mg
B_6	7 mg
B_{12}	40 µg
Biotine	130 µg
Pantothenic acid	26 mg
Choline	1040 mg
Folic acid	1 mg
Nicotinic acid	78 mg
K_3	3 mg

Minerals and trace elements (per kg diet):

Calcium	16 g
Phosphorus	6 g
Sodium	2 g
Magnesium	2 g
Potassium	12 g
Manganese	24 mg
Copper	12 mg
Zinc	24 mg
Iodine	0.2 mg
Iron	120 mg

APPENDIX II - Water

Administration:	ad libitum
System:	drinking nipples
Quality:	drinking water as for human consumption
Quality control:	analytical and bacteriological controls every six months