IBR Forschungs GmbH
Südkampen Nr. 31, D - 3030 Walsrode 1
Telefon (0 51 66) 59-0
Telex 9 24 342 ibr d
Telefax (0 51 66) 50 26

IBR Forschungs GmbH & Co.
Bioanalytisches Zentrum KG
Feodor-Lynen-Straße 5
D - 3000 Hannover 61
Telefon (05 11) 5 30 04 40
Telex 9 24 342 ibr d
Telefax (05 11) 5 30 04 41

October 1990 / wa

FINAL REPORT

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

Project no.: 10-03-0390-90

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

22. NOV. 1990

CONTENTS

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.

The study director accepts overall responsibility for the technical conduct of the study as well as for the interpretation, analysis, documentation and reporting of the results.

Dr. med. vet. J. Lindena

Scientific supervisor

K. Kaufmann

Study director

19.11.90

Date

GLP COMPLIANCE

To the best of my knowledge, this study was performed in accordance with the principles of Good Laboratory Practice for the testing of chemicals as specified by national (BGBl. I, No. 13, § 19 a, March 22, 1990) and international (OECD, Paris, 1982) legislation.

Study Director:

K. Kaufmann

19.11.90

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 natioal (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 audits were reported to management and to the study director.

.....

(p.p. Quality Assurance Unit)

31.10.1990

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 skin. Information derived from this test serves to indicate the existence of possible hazards likely to arise from exposure of the skin to the test article.

The test was conducted according to the OECD guideline for the testing of chemicals (OECD 404, May 12, 1981) and the EEC directive 84/449 EEC. The principles of Good Laboratory Practice for the testing of chemicals as specified by national (BGB1. I, no 13, § 19a, March 22, 1990) and international (OECD, Paris, 1982) legislation were followed during the performace of the study; the IBR standard study protocol, approves and signed an February 28 and March 8, 1990, and the corresponding addendum, signed on February 26, 1990, were valid.

II. SUMMARY

The potential toxicity of **"Koleston 2000 mit 3% Lehmannblausulfat"** was assessed in an acute dermal irritation/corrosion test on 3 albino rabbits. The skin was exposed to the test substance for 4 h. Animals were examined for signs of erythema and oedema at 30-60 min, 24, 48 and 72 h post exposure period and thereafter once daily up to day 13.

Classification

According to the criteria for the evaluation of skin reactions specified by the EEC directive 83/467/EEC from July 29, 1983 and the Gefahrstoffverordnung (GeffStoffV), 1987 (BGBl. I, p. 2721), the present observations provide no evidence of either corrosion or irritation. When applied to the skin, the test article

"Koleston 2000 mit 3% Lehmannblausulfat"

may therefore be classified as **"not irritant"**.

III. MATERIAL AND METHOD

1 Animals

Species:	rabbit
Strain:	White New Zealands
Source:	Harald Schriever, Kaninchenfarm, D-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:	Wella AG Berliner Allee 65 D-6100 Darmstadt
Chemical name:	not specified by sponsor
Physical state:	not specified by sponsor
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
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, fur was removed with electric clippers from an area of roughly 8 x 15 cm on the back of each animal. The skin was subsequently examined for abrasions and animals with healthy, intact skin were then earmarked for individual identification.

Preparation of the Test Article

The test article was applied undiluted.

Procedure

In each animal, 0.5 g of the test article were applied to the test site (ca. 6 cm^2 in size), an adjacent area of untreated skin serving as a control. Each test area was covered with a semi-occlusive dressing consisting of Kosmoplast^R (Medilog), which was held in place by non-irritating tape Elastoplast^R (Beiersdorf AG, Hamburg), and Stülpa^R (P. Hartmann AG, Heidenheim /Brenz), which enveloped the whole of the animal's trunk. At the end of the 4h exposure period, the dressing was removed and any residual sample was carefully washed away with water or an appropriate solvent.

Observations

Signs of erythema and oedema were recorded at 30-60 min, 24, 48 and 72 h after patch removal. If necessary, observations were extended in order to determine the reversibility or irreversibility of the lesions. Dermal irritation was evaluated according to the scheme shown presented on the next page.

Classification

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

EVALUATION OF SKIN REACTION

(as stipulated by OECD 404)

Erythema and Eschar Formation	Value
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

Maximum possible = 4

Oedema Formation	Value
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well defined by definite raising)	2
Moderate oedema (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond area of exposure)	4

Maximum possible = 4

IV. RESULTS

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

Table 1: Individual values of skin reactions

Animal number	Time after patch removal: 30-60min Ery Oed T C T C	1 day Ery Oed T C T C	2 days Ery Oed T C T C	3 days Ery Oed T C T C	4 days Ery Oed T C T C	5 days Ery Oed T C T C	6 days Ery Oed T C T C
1	1 0 1 0	1 0 1 0	1 0 1 0	2 0 1 0	2 0 0 0	2 0 0 0	1 0 0 0
2	1 0 1 0	1 0 1 0	1 0 1 0	3 0 1 0	2 0 0 0	2 0 0 0	1 0 0 0
3	1 0 1 0	1 0 1 0	1 0 1 0	2 0 1 0	0 0 0 0	0 0 0 0	0 0 0 0

Animal number	Time after patch removal: 7 days Ery Oed T C T C	8 day Ery Oed T C T C	9 days Ery Oed T C T C	10 days Ery Oed T C T C	11 days Ery Oed T C T C	12 days Ery Oed T C T C	13 days Ery Oed T C T C
1	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
2	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
3	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0

Table 2: Mean values of skin reactions

	Time after patch removal: 30-60 min	24 h	48 h	72 h
Mean Ery	1.00	1.00	1.00	2.33
Mean Oed	1.00	1.00	1.00	1.00
Mean Ery + Mean Oed	1.00	1.00	1.00	1.66

T = test site

C = control site

Ery = erythema

Oed = oedema

Table 3: Mean values of skin reactions at 24, 48 and 72 h after treatment

Animal number	Erythema (test site)	Oedema (test site)
1	1.33	1.00
2	1.67	1.00
3	1.33	1.00

Test article - dependent findings

Treated areas showed small black alterations of the skin (1 cm x 1 cm).

Classification

According to the criteria for the evaluation of skin reactions specified by the EEC directive 83/467/EEC from July 29, 1983 and the Gefahrstoffverordnung (GeffStoffV), 1987 (BGBl. I, p. 2721), the present observations provide no evidence of either corrosion or irritation. When applied to the skin, the test article

"Koleston 2000 mit 3% Lehmannblausulfat"

may therefore be 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, I. 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), Dr. A. Stöcker (Veterinarian), A. Schweneker (Technician), D. Goretzky (Technician).
Experimental period:	July 26 - August 8, 1990.
Archives:	All raw data, documentation and specimens, the protocol, 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 retained 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 reserves 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

Bezirksregierung Lüneburg
- 205.3-41401/3 -

GLP-Zertifikat

Bescheinigung

Hiermit wird bestätigt, daß
die IBR Forschungs GmbH
in 3030 Walsrode 2,
Südkampen Nr. 31

am 15.04.1988

von der für die Überwachung
zuständigen Behörde nach
Maßgabe der OECD-Richtlinien
für nationale Inspektionen
und Überprüfungen über die
Einhaltung der Grundsätze
der Guten Laborpraxis
inspiziert worden ist.

Es wird hiermit bestätigt, daß
Prüfungen in dieser Prüfein-
richtung nach den OECD-Grund-
sätzen für Gute Laborpraxis
durchgeführt werden.

Certificate

It is hereby certified that
the IBR Forschungs GmbH
in 3030 Walsrode 2,
Südkampen Nr. 31

on 15.04.1988

was inspected by the competent
authority in accordance with
the OECD-Guidelines for
National GLP Inspections
and Study Audits regarding
the compliance with the
Principles of Good Laboratory
Practice.

It is hereby certified that
studies in this test facility
are conducted in compliance
with the OECD-Principles of
Good Laboratory Practice.

Lüneburg, 27. 4. 1988

Im Auftrage

Seippel

Seippel
Pharmaziedirektor

BEZIRKSREGIERUNG LÜNEBURG 70