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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%	Nonylphenoether
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 <sub>2</sub> SO <sub>3</sub> 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

f. Hübner

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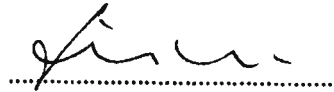
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## DECLARATION

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.

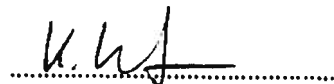
The study director accepts overall responsibility for the technical conduct of the study as well as for the interpretation, analysis, documentation and reporting of the results.

Dr. med. vet. J. Lindena



Scientific supervisor

K. Kaufmann



Study director

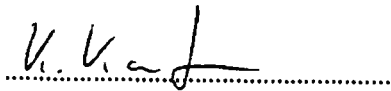
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Date

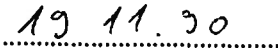
## GLP COMPLIANCE

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 (BGBI. I, No. 13, § 19 a, March 22, 1990) and international (OECD, Paris, 1982) legislation.

Study Director:

A handwritten signature in black ink, appearing to read 'K. Kaufmann', is written over a horizontal dotted line.

K. Kaufmann

A handwritten date '19.11.90' is written in black ink over a horizontal dotted line.

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 audits were reported to management and to the study director.

*L. Schwaneke*  
.....  
(p.p. Quality Assurance Unit)

*31.10.1995*  
.....  
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 performance of the study; the IBR standard study protocol, approved and signed on 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<sup>2</sup> 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<sup>R</sup> (Medilog), which was held in place by non-irritating tape Elastoplast<sup>R</sup> (Beiersdorf AG, Hamburg), and Stülpa<sup>R</sup> (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)

<b>Erythema and Eschar Formation</b>	<b>Value</b>
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	

<b>Oedema Formation</b>	<b>Value</b>
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	30-60min		1 day		2 days		3 days		4 days		5 days		6 days	
	Ery	Oed	Ery	Oed	Ery	Oed	Ery	Oed	Ery	Oed	Ery	Oed	Ery	Oed
	T	C	T	C	T	C	T	C	T	C	T	C	T	C
1	1	0	1	0	1	0	2	0	2	0	2	0	1	0
2	1	0	1	0	1	0	3	0	2	0	2	0	1	0
3	1	0	1	0	1	0	2	0	0	0	0	0	0	0

Animal number	7 days		8 day		9 days		10 days		11 days		12 days		13 days	
	Ery	Oed	Ery	Oed	Ery	Oed	Ery	Oed	Ery	Oed	Ery	Oed	Ery	Oed
	T	C	T	C	T	C	T	C	T	C	T	C	T	C
1	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
3	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 (GefStoffV), 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. Schwenecker (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<sup>R</sup>  
(Alleindiät für Zuchtkaninchen)

Form: pellets, 1.0-1.5 cm long, 0.5 cm diameter

Composition (per kg diet):		Vitamins (supplemented per kg diet):	
Crude protein	160 g	A	13000 IU
Crude fat	25 g	D <sub>3</sub>	1300 IU
Crude fiber	170 g	E	39 mg
Crude ash	100 g	B <sub>1</sub>	8 mg
		B <sub>2</sub>	19 mg
		B <sub>6</sub>	7 mg
		B <sub>12</sub>	40 µg
		Biotine	130 µg
		Pantothenic acid	26 mg
		Choline	1040 mg
		Folic acid	1 mg
		Nicotinic acid	78 mg
		K <sub>3</sub>	3 mg
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		

#### 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**

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



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

