LABORATORY OF PHARMACOLOGY
AND TOXICOLOGY

LPT

ACUTE SKIN IRRITATION/CORROSION TEST (PATCH-TEST) OF NACOL 22 RD IN THE RABBIT

November 17th, 1986

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Directors: Prof. F. Leuschner M.D., B.-W. Neumann Dr. med. vet.

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MANAGEMENT STATEMENT

ACUTE SKIN IRRITATION/CORROSION TEST (PATCH-TEST)
OF NACOL 22 RD IN THE RABBIT

The management hereby certifies that, in the course of controls conducted by the QAU on quality, integrity and correspondence to protocol, no findings were revealed which inhibit the scientific judgement of this study.

Hamburg, November 17th, 1986

Prof.Dr.med.F.Leuschner

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QAU-STATEMENT

Based on a quality assurance review, it was concluded that this report accurately reflects the data for:

ACUTE SKIN IRRITATION/CORROSION TEST (PATCH-TEST)
OF NACOL 22 RD IN THE RABBIT

Performance of study according to "Nonclinical Laboratory Studies. Good Laboratory Practice Regulations". F.R. Vol. 43, No. 247 dated December 22nd, 1978 and according to Good Laboratory Practice Regulations of the OECD and Japan.

Approved and submitted by

F. Hübscher
Director of Quality
Assurance Unit (QAU)

24.11.86
Date

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ACUTE SKIN IRRITATION/CORROSION TEST (PATCH-TEST)
OF NACOL 22 RD IN THE RABBIT

Signature list of scientists and further professionals involved in this experiment/date:

Study Director: 17.11.86
(Dr.med.vet.B.-W.Neumann)

Study Supervision: 17.11.86
(Prof.Dr.med.F.Leuschner)

Conduct of Study/
Animal Husbandry: 17.11.86
(R.Klie)

Quality Assurance Unit
(QAU): 17.11.86
(F.Hübscher)

GENERAL INFORMATION

Test compound:	designation: NACOL 22 RD
Sponsor:	CONDEA CHEMIE GmbH Postfach 1160 D-2212 Brunsbüttel
Testing facilities:	Laboratory of Pharmacology and Toxicology Prof.Dr.med.F.Leuschner Redderweg 8 D-2104 Hamburg 92
Conduct of study:	September/October 1986
Date of report:	November 17th, 1986
Study director:	Dr.med.vet.B.-W.Neumann
Study supervision:	Prof.Dr.med.F.Leuschner
Conduct of study/ Animal husbandry:	R.Klie
Quality-Assurance- Unit (QAU):	F.Hübscher
Species/strain/breeder:	Rabbit/White Russian/ CHR.F.LEUSCHNER & Co.
Reason for of selection of species:	international recommendations
Duration of study:	1 quarantine weeks, 1 test day and at least 72 hours recovery period
Identification of animals:	by tattoo and cage number
Technical conduct:	according to S.O.P.s

Archive of data, raw materials and samples of test compound:	during the course of the study: archive 1 and I Laboratory of Pharmacology and Toxicology Prof.Dr.med.F.Leuschner Redderweg 8 D-2104 Hamburg 92 after reporting: biological material in the archive Mienenbüttel written raw data: in the archive Löhndorf copy of final report: archive I Redderweg 8 D-2104 Hamburg 92
Protocol for this report:	dated June 13th, 1986

1. INTRODUCTION

In this experiment the tolerance of healthy intact rabbit skin to NACOL 22 RD, batch 9153 - called 'NACOL 22' - was examined.

2. METHODS

The study was performed basing on OECD-method 404 and according to US and Japanese GLP-regulations and the EEC directive dated April 25th, 1984.

Principle: 0.5 g of the test substance is applied to the shaved intact dorsal skin of rabbits (area: 2.5 x 2.5 cm). The skin is examined 30 to 60 minutes and then 24, 48 and 72 hours after a 4-hour exposure period. If lesions occur, observations are continued until complete recovery.
In addition, behaviour, general condition and food consumption are observed in the main experiment.

2.1. Animals:

In this experiment 3 male white Russian rabbits (breeder: CHR.LEUSCHNER & Co., D-2355 Löhndorf) were used. Their initial body weight was 2.2 - 2.3 kg, their age approximately 7 months.

2.2. Dose level and administration:

The patch test was used for this experiment. 0.5 g of the test compound was applied to a linen patch (size: 2.5 x 2.5 cm). This patch was covered with plastic foil of the same size, placed on the shaved intact dorsal skin of the animals and secured with an adhesive plaster (LEUKOPLAST, Beiersdorf & Co. AG, Unnastr. 48, D-2000 Hamburg) between the fore and hind limbs.
The linen patch remained on the skin for 4 hours. During this period the animals were kept in restrainers.

2.3. Skin evaluation:

After 4 hours of exposure, patches were removed and the resulting reactions examined. Assessments were made 30 to 60 minutes and 24, 48 and 72 hours after patch removal. If necessary, observations are repeated until complete recovery.

Skin reactions were evaluated by two independent experts. Where assessments differ, the mean value is taken.

Evaluations of skin irritation are based on the following scheme by DRAIZE:

Erythema

0	=	no erythema
1	=	very slight erythema (barely perceptible)
2	=	well-defined erythema
3	=	moderate to severe erythema
4	=	severe erythema (beet-coloured) to slight eschar formations (injuries in depth)

Oedema

0	=	no oedema
1	=	very slight oedema (barely perceptible)
2	=	slight oedema (edges of area well defined by definite raising)
3	=	moderate oedema (raised approx. 1 mm)
4	=	severe oedema (raised more than 1 mm and extending beyond area of exposure)

2.4. Evaluation:

Reactions of the intact skin are evaluated 30 to 60 minutes and then 24, 48 and 72 hours after patch removal, as described before. Where reactions are found at any of these time periods, primary irritation is calculated in the following way:

The assessments of the erythema-/eschar-formation on intact skin taken from observations made 30 to 60 minutes, 24, 48 and 72 hours after patch removal were added together (a total of 4 values).

The assessments of the oedema formation taken from observations made 30 to 60 minutes, 24, 48 and 72 hours after patch removal (a total of 4 values) were added together.

The sum of these 8 figures was divided by 4, resulting in the degree of lesion (= degree of primary irritation):

primary irritation range	evaluation scheme
0.0 - 0.5	no to very slight irritation
0.6 - 3.0	slight irritation
3.1 - 5.0	moderate irritation
5.1 - 8.0	severe irritation

Should further lesions occur, they are listed.

2.5 Diet and housing:

Artificial diet ALTROMIN 2023 (supplied by: ALTROMIN GmbH, P.O.Box 285, D-4937 Lage/Lippe, composition see next page) served as food.
Periodic analyses of food for contaminants are carried out at least twice a year by the Landwirtschaftliche Untersuchungs- und Forschungsanstalt Kiel, D-2300 Kiel 1. ALTROMIN was offered ad lib.
Tap water was also given unrestrictedly and is examined for contaminants at least once a year (Wasserbeschaffungsverband Harburg, Staatliches Chemisches Untersuchungsamt Lüneburg, Stresemannstr. 20, D-2120 Lüneburg).

All analyses are performed according to the EPA/USA, PROPOSED HEALTH EFFECTS TEST STANDARDS FOR TOXIC SUBSTANCES CONTROL ACT TEST RULES, Federal Register Vol. 44, pp. 27334 - 27375, May 1979. Results were within the admissible limits.

During the observation period the animals were kept separately in stainless steel cages with a floor space of 0.4 m^2. Room temperature was 20° ± 3°C (maximum range). Animal cages were lit from 6 a.m. to 6 p.m. (about 150 lux at 1.50 m room height) and darkened from 6 p.m. to 6 a.m.

2.6. Additional examinations:

In addition to the observation of the skin, behaviour, general condition and food consumption were examined daily after patch removal. Body weight was recorded daily.

2.7. Experimental period:

This experiment was carried out in September/October 1986.

Standard Diet for Rabbits

ALTROMIN 2023

(Altromin GmbH, D-4937 Lage/Lippe)

raw nutrient media (% in the diet)	
raw protein	17.5
raw fat	4.0
raw fibres	14.5
ash	9.0
water	12.0
non-nitrogenous extractive substances	43.0
realizable energy:	
kcal/kg	2 700.0
kJ/kg	11 300.0

amino acids (% in the diet)	
lysine	0.80
methionine	0.30
cystine	0.20
phenylalanine	0.80
tyrosine	0.50
arginine	0.90
histidine	0.40
tryptophane	0.20
threonine	0.60
isoleucine	0.90
leucine	1.30
valine	0.90

minerals (% in the diet)	
calcium	1.0
phosphorus	0.7
magnesium	0.2
sodium	0.2
potassium	1.6

trace elements (mg in 1000 g of diet)	
manganese	60.0
iron	160.0
copper	17.0
zinc	50.0
iodine	0.9
fluorine	10.0

vitamins
(admixture per 1000 g of diet)

vitamin A	15 000 IE
vitamin D_3	600 IE
vitamin E	75 mg
vitamin K_3	3 mg
vitamin B_1	18 mg
vitamin B_2	12 mg
vitamin B_6	9 mg
vitamin B_{12}	24 µg
nicotinic acid	36 mg
pantothenic acid	21 mg
folic acid	2 mg
biotin	60 µg
choline	600 mg
vitamin C	36 mg
iron	

3. RESULTS

Local reaction:

After single application of 0.5 g NACOL 22 onto the intact dorsal skin of rabbits no damages to the skin were observed after the removal of the patch at the end of 4 hours of exposure as well as after 4.5, 5, 28, 52 and 76 hours.

Individual results are to be found in the following tables.

General (systemic) reaction:

No systemic intolerance reactions were observed after the single application of 0.5 g NACOL 22 onto the intact dorsal skin of rabbits. The animals' behaviour and general condition, their care of fur, food consumption and body weight gain were normal.

TABLE

Examination on primarily irritating properties in rabbit skin
single administration (patch test)
individual findings and mean values from 3 animals

Findings	Date after 4-hour exposition	Skin reaction Animal no. 1	2	3	Mean value	Primarily irritating properties
	0.5 g NACOL 22/animal					
erythema/ eschar formation	30 - 60 minutes	0	0	0	0	0
	24 hrs	0	0	0	0	0
	48 hrs	0	0	0	0	0
	72 hrs	0	0	0	0	0
oedema	30 - 60 minutes	0	0	0	0	0
	24 hrs	0	0	0	0	0
	48 hrs	0	0	0	0	0
	72 hrs	0	0	0	0	0
					total:	0

degree of lesion: 0 = no irritating properties