LABORATORY OF PHARMACOLOGY
AND TOXICOLOGY

LPT

EYE IRRITATION STUDY OF NACOL 22 RD IN THE RABBIT AFTER SINGLE INSTILLATION INTO THE CONJUNCTIVAL SAC

November 18th, 1986

Redderweg 8, D-2104 Hamburg 92 (West Germany), Telephone: (040) 701 50 21-23, Telex: 2165349 lpt d
Directors: Prof. F. Leuschner M.D., B.-W. Neumann Dr. med. vet.

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MANAGEMENT STATEMENT

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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 18th, 1986

Prof.Dr.med.F.Leuschner

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QUALITY ASSURANCE STATEMENT

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

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The quality assurance review in question was performed according to 'Nonclinical Laboratory Studies. Good Laboratory Practice Regulations' (FDA/USA), F.R. Vol. 43, No. 247, dated December 22nd, 1978 as well as in compliance with 'Good Laboratory Practice'-Regulations of the OECD and Japan.

Approved and submitted by: F. Hübscher — 25.11.86

F. Hübscher
Director of Quality
Assurance Unit (QAU)

Date

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

Study director: 18.11.86
Dr.med.vet.B.-W.Neumann

Study supervision: 18.11.86
Prof.Dr.med.F.Leuschner

Conduct of study: 18.11.86
R.Klie

Quality assurance unit (QAU): 18.11.86
F.Hübscher

GENERAL INFORMATION

Test compound: Batch no.:	NACOL 22 RD 9153 - called for short 'NACOL 22' -
Sponsor:	CONDEA CHEMIE GmbH Postfach 1160 D-2212 Brunsbüttel 1
Testing facilities:	Laboratory of Pharmacology and Toxicology Prof.Dr.med.F.Leuschner Redderweg 8 D-2104 Hamburg 92
Conduct of study:	October 1986
Date of report:	November 18th, 1986
Study director:	Dr.med.vet.B.-W.Neumann
Study supervision:	Prof.Dr.med.F.Leuschner
Conduct of study:	R.Klie
Quality assurance unit (QAU):	F.Hübscher
Animal species/strain/ breeder:	rabbit/white Russian/ CHR.F.LEUSCHNER & CO. D-2355 Löhndorf
Reason for selection of species:	international recommendations
Duration of study:	1 quarantine week, administration and a subsequent recovery period (72 hours)
Identification of animals:	by ear tattoo and cage number
Archive of data, raw materials and samples of test compound:	during the study: archives 1 and I Laboratory of Pharmacology and Toxicology 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
Technical conduct:	according to SOPs
Protocol for this report:	dated June 13th, 1986

1. INTRODUCTION

The aim of this experiment was to obtain information on the tolerance of rabbit eyes to NACOL 22 RD, batch 9153 - called for short 'NACOL 22' - after single application into the conjunctival sac.

2. METHODS

The study based on the OECD-method 405 and the GLP regulations of the USA, the OECD and Japan and the EEC directive dated April 25th, 1984.

2.1. Animals:

In this experiment 3 rabbits (white Russian, breeder CHR. F.LEUSCHNER & Co., D-2355 Löhndorf) with an initial weight of 2.10 to 2.34 kg were used. Initially age of the rabbits was approximately 7 months.

No animals with eye diseases were treated.

2.2. Dosage and administration:

100 mg NACOL 22 were administered in pulverized form once into the conjunctival sac of the left eye. As a control the right eye was treated with 0.1 ml of 0.9% NaCl-solution[1].

For the application the rabbit was held down but in such a way that the animal did not feel uncomfortable. Then the lower eyelid was carefully pulled away from the eyeball and the test compound inserted into the conjunctival sac. Afterwards the eyelid was closed for 1 second and then the animal was set free. The eyes were not rinsed.

[1] supplied by: BRAUN MELSUNGEN AG, D-3508 Melsungen

2.3. Diet and housing:

Artificial diet for rabbits ALTROMIN 2023 (supplied by: ALTROMIN GmbH, D-4937 Lage/Lippe, composition: see next page) served as food. Food and tap water were offered ad lib. Periodic analyses of the diet for contaminants are carried out at least twice a year by the Landwirtschaftliche Untersuchungs- und Forschungsanstalt Kiel, Gutenbergstr. 75 - 77, D-2300 Kiel 1. Samples of drinking water are taken once a year by the Wasserbeschaffungs-Verband Harburg, D-2105 Seevetal 1, and are analysed by the 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. The results were within the admissible limits.

During the observation period the animals were kept separately in V_2A-steel cages (base: 0.4 m^2). Room temperature was maintained at 20° ± 3°C (maximum range). The cages were lit from 6 a.m. to 6 p.m. (150 lux at 1.50 m room height) and darkened from 6 p.m. to 6 a.m. The metabolism cages were such that irritation of the eyes from excrement and urine could be excluded.

Standard Diet for Rabbits

ALTROMIN 2023

(ALTROMIN GmbH, D-4937 Lage/Lippe)

Ingredients (average % content in the diet)	
crude protein	17.5
crude fat	4.0
crude fibres	14.5
ash	9.0
moisture	12.0
nitrogen-free extract	43.0

Metabolizable Energy:	
(kcal/kg)	2700.0
(kJ/kg)	11300.0

Amino Acids (average % content 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 (average % content in the diet)	
calcium	1.0
phosphorus	0.7
magnesium	0.2
sodium	0.2
potassium	1.6

Trace Elements (average content in mg per 1000 g of diet)	
manganese	60.0
iron	160.0
copper	17.0
zinc	50.0
iodine	0.9
fluorine	10.0

Vitamins

(additive per 1000 g of diet)

vitamin A	15000.0 IU
vitamin D_3	600.0 IU
vitamin E	75.0 mg
vitamin K_3	3.0 mg
vitamin B_1	18.0 mg
vitamin B_2	12.0 mg
vitamin B_6	9.0 mg
vitamin B_{12}	24.0 µg
nicotinic acid	36.0 mg
pantothenic acid	21.0 mg
folic acid	2.0 mg
biotin	60.0 µg
choline	600.0 mg
vitamin C	36.0 mg

2.4. Examinations:

Prior to administration the eyes were examined ophthalmologically with a slit lamp and after 5, 15, 30 minutes as well as 1, 2, 4, 24, 48 and 72 hours after application. Two independent experts observed the eye reactions. Where results deviated the mean values were taken.

24 hours after administration the eyes were treated with fluorescein (fluorescein-sodium-solution USP) and examined.

Reactions were valued by the following scheme based on DRAIZE:

2.4.1. CORNEA

A) Opacity

0 no opacity

1 scattered or diffuse areas of opacity, details of iris clearly visible

2 not completely translucent areas, details of iris slightly obscured

3 nacreous areas, details of iris not visible, size of pupil barely discernible

4 complete corneal opacity, iris not discernible

B) Involvement

0 no involvement

1 one quarter or less, but not 0

2 exceeding one quarter, but less than half

3 exceeding one half, but less than three quarters

4 exceeding three quarters up to whole area

Evaluation: A x B x 5 Maximum Score: 80

2.4.2. A) IRIS

0 normal

1 markedly deepened folds, congestion, swelling, moderate circumcorneal injection (any of these or combination of any thereof), iris still reacting to light)

2 no reaction to light, haemorrhage, gross destruction (any or all of these)

Evaluation: A x 5 Maximum Score: 10

2.4.3. CONJUNCTIVAE

A) Redness

0 vessels normal

1 hyperaemia

2 diffuse redness, individual vessels not discernible

3 massive redness of all sections

B) Swelling (lids and/or nictitating membrane)

0 no swelling

1 slight swelling (incl. nictitating membrane)

2 externally visible swelling with eversion of lid

3 swelling, which leads to half closed lids

4 swelling, which leads to more than half closed lids up to totally closed lids

C) Secretion

0 no abnormal secretion

1 hypersecretion

2 hypersecretion with moistening of lids and hairs just adjacent to lids

3 hypersecretion with moistening of lids and hairs and considerable area around the eye

Evaluation: (A + B + C) x 2 Maximum Score: 20

The grades of possible changes were classified as follows:

Maximal score (total)	Evaluation
0 - 10	none to very slight irritation
11 - 25	slight irritation
26 - 56	moderate irritation
57 - 110	severe irritation

2.5. Experimental period:

This experiment was carried out in October 1986.

3. RESULTS

Under present test conditions NACOL 22 caused slight erythema and slightly increased secretion of the conjunctivae after the administration of 100 mg into the conjunctival sac of the rabbits's eye. 15 and 30 min. as well as 1 and 2 hours after instillation all animals used showed reddening of the conjunctivae at reaction stage 1 according to DRAIZE, hypersecretion was seen in one animal 15 min. after administration. There were no longer any pathological findings 2 hours after instillation.
Cornea and iris remained unaffected and the fluorescein test carried out 24 hours after instillation revealed no indications of erosions or ulcerations.

Under present test conditions NACOL 22 may be considered to have very slightly irritating properties.

Individual results are to be found in the following tables.

TABLE

Examination on primarily irritating properties in rabbit eyes (in brackets number of affected animals)

Date after instillation	Cornea		Iris	Conjunctivae			Total score according to DRAIZE
	degree A	affected B		redness	swelling	hypersecretion	
				- reaction stages according to DRAIZE -			
	100 mg NACOL 22/eye						
5 min	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0.0
15 min	0 (3)	0 (3)	0 (3)	1 (3)	0 (3)	1 (1) 0 (2)	2.6
30 min	0 (3)	0 (3)	0 (3)	1 (3)	0 (3)	0 (3)	2.0
1 h	0 (3)	0 (3)	0 (3)	1 (2) 0 (1)	0 (3)	0 (3)	1.4
2 hrs	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0.0
4 hrs	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0.0
24 hrs	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0.0
48 hrs	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0.0
72 hrs	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0.0
7 d	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0.0
24 hrs	fluorescein-test: no pathological findings						

maximum score according to DRAIZE: 2.6 = very slight irritating properties