

EYE IRRITATION STUDY OF NACOL 20 IN THE  
RABBIT AFTER SINGLE INSTILLATION  
INTO THE CONJUNCTIVAL SAC

November 18th, 1986

MANAGEMENT STATEMENT

EYE IRRITATION STUDY OF NACOL 20 IN THE  
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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

QUALITY ASSURANCE STATEMENT

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

EYE IRRITATION STUDY OF NACOL 20 IN THE  
RABBIT AFTER SINGLE INSTILLATION  
INTO THE CONJUNCTIVAL SAC

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  
Director of Quality  
Assurance Unit (QAU)

25.11.88  
Date

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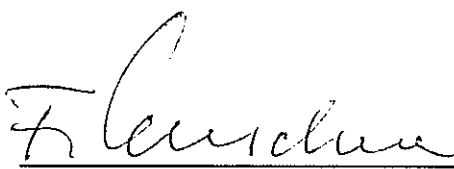
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Signature list of scientists and further professionals  
involved in this study/date:

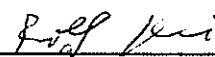
Study director:

 18.11.86  
Dr. med. vet. B.-W. Neumann

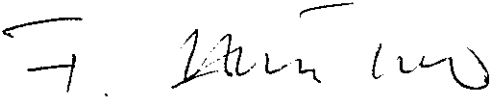
Study supervision:

 7.8.77.86  
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:                                                        | NACOL 20                                                                                                    |
| Batch no.:                                                            | 4601                                                                                                        |
| Sponsor:                                                              | CONDEA CHEMIE GmbH<br>Postfach 1160<br>D-2212 Brunsbüttel 1                                                 |
| Testing facilities:                                                   | Laboratory of Pharmacology<br>and Toxicology<br>Prof.Dr.med.F.Leuschner<br>Redderweg 8<br>D-2104 Hamburg 92 |
| 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<br>unit (QAU):                                      | F.Hübscher                                                                                                  |
| Animal species/strain/<br>breeder:                                    | rabbit/white Russian/<br>CHR.F.LEUSCHNER & CO.<br>D-2355 Löhndorf                                           |
| Reason for selection<br>of species:                                   | international recommendations                                                                               |
| Duration of study:                                                    | 1 quarantine week, administration<br>and a subsequent recovery period<br>(72 hours)                         |
| Identification of<br>animals:                                         | by ear tattoo and cage number                                                                               |
| Archive of data,<br>raw materials and<br>samples of test<br>compound: | during the study:<br>archives 1 and I<br>Laboratory of Pharmacology<br>and Toxicology<br>D-2104 Hamburg 92  |

after reporting:  
biological material  
in the archive Mienenbüttele

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 20 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 20 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<sup>1</sup>.

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.

<sup>1</sup> 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<sub>2</sub>A-steel cages (base: 0.4 m<sup>2</sup>). 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 <sub>3</sub>  | 600.0 IU   |
| vitamin E               | 75.0 mg    |
| vitamin K <sub>3</sub>  | 3.0 mg     |
| vitamin B <sub>1</sub>  | 18.0 mg    |
| vitamin B <sub>2</sub>  | 12.0 mg    |
| vitamin B <sub>6</sub>  | 9.0 mg     |
| vitamin B <sub>12</sub> | 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 20 in pulverized form caused slight changes of the conjunctivae after administration of 100 mg into the conjunctival sac of the rabbits's eye. Reddening of the conjunctivae was observed between 5 min. and up to 4 hours after instillation at reaction stage 1 according to DRAIZE and conjunctival hypersecretion was found between 5 and 15 min. after administration, also at degree 1. No pathological findings were to be seen 24 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 20 may be considered to have very slightly irritating properties.

Individual results are specified in the following tables.

A handwritten signature in dark ink, consisting of several stylized, connected loops and a long horizontal stroke at the end.

TABLE

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

| Date after<br>instillation                                                 | Cornea                                     |          | Iris  | Conjunctivae                               |          |                | Total score<br>according to<br>DRAIZE |
|----------------------------------------------------------------------------|--------------------------------------------|----------|-------|--------------------------------------------|----------|----------------|---------------------------------------|
|                                                                            | degree                                     | affected |       | redness                                    | swelling | hypersecretion |                                       |
|                                                                            | A                                          | B        |       | - reaction stages according to<br>DRAIZE - |          |                |                                       |
| 100 mg NACOL 20/eye                                                        |                                            |          |       |                                            |          |                |                                       |
| 5 min                                                                      | 0 (3)                                      | 0 (3)    | 0 (3) | 1 (2) 0 (1)                                | 0 (3)    | 1 (1) 0 (2)    | 2.0                                   |
| 15 min                                                                     | 0 (3)                                      | 0 (3)    | 0 (3) | 1 (2) 0 (1)                                | 0 (3)    | 1 (1) 0 (2)    | 2.0                                   |
| 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 (3)                                      | 0 (3)    | 0 (3)          | 2.0                                   |
| 2 hrs                                                                      | 0 (3)                                      | 0 (3)    | 0 (3) | 1 (1) 0 (2)                                | 0 (3)    | 0 (3)          | 0.7                                   |
| 4 hrs                                                                      | 0 (3)                                      | 0 (3)    | 0 (3) | 1 (1) 0 (2)                                | 0 (3)    | 0 (3)          | 0.7                                   |
| 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: 2.0 = very slight irritating properties |                                            |          |       |                                            |          |                |                                       |