LABORATORY OF PHARMACOLOGY
AND TOXICOLOGY

LPT

ACUTE TOXICITY - ORAL - OF NACOL 22 RD IN SPRAGUE-DAWLEY RATS

January 15th, 1987

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, January 15th, 1987

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

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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 and EEC Regulations.

Approved and submitted by:

F. Hübscher
Director of Quality
Assurance Unit (QAU)

21.1.87
Date

ACUTE TOXICITY - ORAL - OF NACOL 22 RD
IN SPRAGUE-DAWLEY RATS

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

Study Director: (Prof. Dr. med. F. Leuschner) 27.7.87

Study Supervision: (Dr. med. vet. B.-W. Neumann) 21.1.87

Conduct of Study: (Dipl. Biol. M. Liebsch) 21.1.87

Animal Husbandry: (G. Stehr) 21.1.87

Quality Assurance Unit (QAU): (F. Hübscher) 21.1.87

GENERAL INFORMATION

Test compound:	NACOL 22 RD batch no. 9153 - called for short 'NACOL 22' -
Consistency:	solid plates (pulverized by grinding)
Animal species/ strain/stock:	Rat/Sprague-Dawley/ Tif: RAI f (SPF)
Breeder:	Lippische Versuchstierzucht HAGEMANN GmbH & Co. D-4923 Extertal 1
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
Study director:	Prof.Dr.med.F.Leuschner
Study supervision:	Dr.med.vet.B.-W.Neumann
Conduct of study:	Dipl.Biol.M.Liebsch
Quality Assurance Unit:	F.Hübscher
Date of report:	January 15th, 1987
Archive of data (including analysis results), raw material, samples of test compound:	during the study: Laboratory of Pharmacology and Toxicology Prof.Dr.med.F.Leuschner Redderweg 8 D-2104 Hamburg 92 after reporting: written raw data in archive Löhndorf copy of final report: archive I D-2104 Hamburg 92
Technical conduct:	according to S.O.Ps.
Protocol:	dated September 23rd, 1986

1. INTRODUCTION

The aim of the present experiment was to obtain information on the toxic range and signs of toxicity of NACOL 22 RD, batch 9153 - called for short 'NACOL 22' - (in the case of mortality the cause of death) after a single oral administration to rats.

2. METHOD

Conduct of study basing on OECD-method 401, according to GLP-regulations as well as -proposals of the USA/EEC.

2.1. Animals:

For this experiment male and female Sprague-Dawley rats, Tif: RAI f (SPF) were used (breeder: Lippische Versuchstierzucht HAGEMANN GmbH & Co., D-4923 Extertal 1). The initial weight of the animals was 157 - 167 g, their age was 42 - 50 days. Animals were allocated to the different groups by randomisation.

2.2. Dosage and administration:

NACOL 22 was administered once by stomach tube, 0.8% hydroxypropyl-methylcellulose gel# served as vehicle. Dose levels of 8250 and 10000 mg/kg b.w. were tested in 5 male and 5 female animals for each group. The dose interval followed the factor 1.21.

2.3. Food and accommodation:

Artificial diet for rats ALTROMIN 1324 (supplied by: ALTROMIN GmbH, D-4937 Lage/Lippe, composition see next page) served as food. Feeding was discontinued 15 - 16 hours before administration of dose, only tap water was offered ad lib.

METHOCEL E 4 M, supplied by: Synopharm,
Apotheker Fritz Zimmermann,
Postfach 1205, D-2000 Barsbüttel

Standard Diet for Rats and Mice

ALTROMIN 1324

(ALTROMIN GmbH, D-4937 Lage/Lippe)

Ingredients

(average % content in the diet)

crude protein	19.0
crude fat	4.0
crude fibres	6.0
ash	7.0
moisture	13.5
nitrogen-free extract	50.5

Metabolizable Energy:

(kcal/kg)	3 100.0
(kJ/kg)	13 000.0

Amino Acids

(average % content in the diet)

lysine	0.90
methionine	0.30
cystine	0.30
phenylalanine	0.80
tyrosine	0.60
arginine	1.10
histidine	0.40
tryptophane	0.20
threonine	0.60
isoleucine	0.80
leucine	1.30
valine	0.90

Minerals

(average % content in the diet)

calcium	0.9
phosphorus	0.7
magnesium	0.2
sodium	0.2
potassium	1.0

Trace Elements

(average content in mg per 1000 g of diet)

manganese	75.0
iron	180.0
copper	13.0
zinc	70.0
iodine	0.9
fluorine	9.0

Vitamins

(additive per 1000 g of diet)

vitamin A	15 000.0 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

Periodic analyses of food for contaminants are carried out at least twice a year by the Landwirtschaftliche Untersuchungs- und Forschungsanstalt Kiel, Gutenbergstraße 75 - 77, D-2300 Kiel 1. Tap water is analysed regularly by the Hamburger Wasserwerke, D-2000 Hamburg 1.

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.

The animals were kept separately in MAKROLON cages (type III). Room temperature was 21° ± 1.0° C (maximum range), relative humidity 55% ± 5% (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.

2.4. Evaluation:

The animals were observed for a period of 14 days after administration. During this time behaviour, food and drinking water consumption as well as body weight gain were observed. At the end of the experimental period all animals were sacrificed, dissected and inspected macroscopically.

2.5. Experimental period:

This experiment was carried out in October/November 1986.

3. RESULTS

After single oral administration of NACOL 22 no intolerance reactions occurred up to the highest tested dose-level of 10000 mg/kg b.w. orally. Higher tested dosages were not suitable because of physiological reasons.
A summary of the results is listed in Table 1. Table 2 informs on individual results.

TABLE 1

Summarised Results
Acute toxicity - oral - NACOL 22 in rats

Substance	Number of Animals Sex	LD_{50} (24 hours) mg/kg b.w. p.o.	LD_{50} (14 days) mg/kg b.w. p.o.	Lowest Toxic Dose-level mg/kg b.w. p.o.	Toxic Signs
NACOL 22	10 m	> 10000#	> 10000#	~ 10000	No signs of intolerance reactions up to the highest tested reasonable dose-level (10000 mg/kg b.w. orally = maximum administration volume of the undiluted test preparation). Autopsy: no pathological findings
	10 f	> 10000#	> 10000#	~ 10000	

no LD_{50} could be determined because highest reasonable dose-level without toxic signs and mortality

m = male animals
f = female animals

TABLE 2

Detailed Results
Acute toxicity - oral - NACOL 22 in rats

Dose-level in mg/kg b.w. p.o.	No. of Animals	Toxic Signs	Inhibition of Food Intake in %# 1 2 7 Test day			Inhibition of Body Weight Gain in %# 1 2 7 Test day			Number of Animals Which Died 24 h / 14 days m f m f			
8250	5 m 5 f	No signs of intolerance reactions. Autopsy: no pathological findings.	0	0	0	0	0	0	0	0	0	0
10000	5 m 5 f	No signs of intolerance reactions. Autopsy: no pathological findings.	0	0	0	0	0	0	0	0	0	0

# mean value of the group
m = male
f = female

3. ERGEBNISSE

Unter den vorliegenden Versuchsbedingungen verursachte NACOL 22 nach Verabreichung von 100 mg in den Konjunktivalsack des Kaninchenauges leichtes Erythem und leicht gesteigerte Sekretion der Konjunktiven. Die Rötung fand sich bei allen eingesetzten Tieren 15 und 30 min sowie 1 und 2 Stunden nach der Instillation in Reaktionsstufe 1 nach DRAIZE, die Hypersekretion in einem Fall 15 min nach der Gabe. 2 Stunden nach der Instillation konnten keine pathologischen Befunde mehr erhoben werden.
Cornea und Iris blieben unbeeinträchtigt, beim Fluorescein-Test 24 Stunden nach der Instillation ergaben sich keine Hinweise auf Erosionen oder Ulcerationen.

Unter den vorliegenden Versuchsbedingungen besitzt NACOL 22 geringfügig irritierende Eigenschaften.

Die Einzelergebnisse sind der folgenden Tabelle zu entnehmen.

TABELLE

Untersuchung auf primär irritierende Wirkung am Kaninchenauge
In Klammern Anzahl der betroffenen Tiere

Zeitpunkt nach der Applikation	Cornea		Iris	Konjunktiven			Gesamtzahl nach DRAIZE
	Grad A	Umfang B		Rötung	Schwellung	Sekretion	
	- Reaktionsstufen nach DRAIZE -						
	100 mg NACOL 22/Auge						
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 Std.	0 (3)	0 (3)	0 (3)	1 (2) 0 (1)	0 (3)	0 (3)	1.4
2 Std.	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0.0
4 Std.	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0.0
24 Std.	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0.0
48 Std.	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0.0
72 Std.	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0.0
7 Tagen	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0.0
24 Std.	Fluorescein-Test: keine pathologischen Befunde						

Maximalbewertung nach DRAIZE: 2.6 = geringfügig irritierende Eigenschaften