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August 27, 2002

Attn: TSCA Section 8(e) Coordinator
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U.S. Environmental Protection Agency
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Washington, D.C. 20460

**Celanese Chemicals**
EHSA/Product Stewardship

1601 West LBJ Freeway
Dallas, TX 75234-6034

Debra Phillips
Phone: 972-443-4703
Fax: 972-443-8595
dphillips@celanese.com

CERTIFIED MAIL RECEIPT # Z 300 431 697

**Ref.: TSCA 8(e) Submission – N,N-di-tertbutylethylenediamine (CAS# 4062-60-6)**
**N,N-dimethyl-dipropylenetriamine (CAS# 10563-29-8)**
**Isononylamine (CAS# 27775-00-4)**

**This submission contains no confidential business information.**

Contain NO CBI

Dear Sir or Madam,

In accordance with the requirement of TSCA Section 8(e), Celanese, Ltd. hereby submits reports for acute oral toxicity studies in rats with N,N-di-tertbutylethylenediamine (CAS# 4062-60-6), N,N-dimethyl-dipropylenetriamine (CAS#10563-29-8), and Isononylamine (CAS# 27775-00-4). In each study, clinical signs possibly indicative of neurotoxicity were reported.

The acute oral LD50 in rats with N,N-di-tertbutylethylenediamine was 477.7 mg/kg. In this study, clinical signs possibly indicative of neurotoxicity (e.g., ataxia (muscular incoordination) and muscular spasms) were reported in some surviving animals at the 178 mg/kg and/or 562 mg/kg doses for periods extending from one hour post dose to study Day 14.

The acute oral LD50 in rats with N,N-dimethyl-dipropylenetriamine is 1710 mg/kg. In this study, clinical signs possibly indicative of neurotoxicity (e.g., ataxia, ptosis and loss of righting reflex) were reported in some surviving animals for periods extending through 3 days after dosing with 1000 mg/kg or 1710 mg/kg. A Russian study cited in the U.S. RTECS database mentions behavioral effects of "depressed activity & excitement" in an "unspecified" species in an intraperitoneal LD50 study and an oral LD50 study.

With Isononylamine, the acute oral LD50 in male rats was in the range: 315-500 mg/kg. The acute oral LD50 for female rats is similar, since 40% mortality was observed in a group of female rats tested at 400 mg/kg. In this study, clinical signs possibly indicative of neurotoxicity (e.g., impaired motility, decreased spontaneous activity, squatting posture, stilted/uncoordinated/ataxic gait, stupor, straddling hindlimbs, trembling, tonic as well as saltatory & rolling convulsions) were reported in some surviving animals for periods extending through 3 days after dosing with 25 mg/kg to 400 mg/kg.

If any further information is required, do not hesitate to contact Debra Phillips, Coordinator, Product Stewardship at 972-443-4703.

Sincerely,

Debra Phillips

Debra Phillips
Coordinator, Product Stewardship
Celanese

8EHQ-02-15190

Enclosure

88020000176

01700

# ACUTE ORAL TOXICITY STUDY IN RATS

# FOUR COMPOUNDS

# FINAL REPORT

Submitted to

Virginia Chemicals Inc.
Portsmouth, Virginia

HAZLETON LABORATORIES AMERICA, INC.
9200 Leesburg Turnpike • Vienna, Virginia 22180 • U.S.A.

February 6, 1976

HAZLETON LABORATORIES AMERICA, INC.

SPONSOR: Virginia Chemicals Inc.

DATE: February 6, 1976

MATERIAL: N,N'-1,2-Ethanediylidenebis-t-Butylamine(Diimine)
N,N-Dimethyl-N-Butylamine
N,N-Dimethylethylamine
N,N-Di-tertbutylethylenediamine

SUBJECT: FINAL REPORT
Acute Oral Toxicity Study in Rats
Project No. 533-113

## SUMMARY

Diimine, N,N-Dimethyl-N-Butylamine, N,N-Dimethylethylamine, and N,N-Di-tertbutylethylenediamine were evaluated for acute oral toxicity in male rats. The acute oral $LD_{50}$ for Diimine was calculated to be 1731.8 mg/kg of body weight; for N,N-Dimethyl-N-Butylamine to be 187.8 mg/kg of body weight; for N,N-Dimethylethylamine to be 593.4 mg/kg of body weight; and for N,N-Di-tertbutylethylenediamine to be 477.7 mg/kg of body weight.

## TEST MATERIAL

### Identification and Description

N,N'-1,2-Ethanediylidenebis-t-Butylamine (Diimine) - A white powder with a disagreeable odor.

N,N-Dimethyl-N-Butylamine, N,N-Dimethylethylamine, and N,N-Di-tertbutylethylenediamine were clear liquids with disagreeable odors.

### Date Received

All chemicals were received on December 1, 1975.

## METHODS

One hundred and twenty-five male albino rats of the Sprague-Dawley cesarean-derived strain (received from Charles River Laboratories, Wilmington, Massachusetts) were assigned to the following groups and fasted overnight prior to treatment.

| Compound | No. of Animals | Dose Level mg/kg | Dose Volume ml/kg | Concentration mg/ml |
|---|---|---|---|---|
| Diimine | 5 | 1000 | 5.00 | 200 |
| | 5 | 1470 | 7.35 | 200 |
| | 5 | 1780 | 8.90 | 200 |
| | 5 | 2150 | 10.75 | 200 |
| | 5 | 3160 | 15.80 | 200 |
| | 5 | 4640 | 23.20 | 200 |
| | 5 | 10,000 | 50.00 | 200 |
| N,N-Dimethyl-N-Butylamine | 5 | 100 | 2.00 | 50 |
| | 5 | 178 | 1.19 | 150 |
| | 5 | 316 | 2.11 | 150 |
| | 5 | 562 | 3.75 | 150 |
| | 5 | 1000 | 1.38 | 726 |
| | 5 | 10,000 | 13.77 | 726 |
| N,N-Dimethylethylamine | 5 | 100 | 2.00 | 50 |
| | 5 | 178 | 1.19 | 150 |
| | 5 | 316 | 2.11 | 150 |
| | 5 | 562 | 3.75 | 150 |
| | 5 | 1000 | 1.50 | 667 |
| | 5 | 10,000 | 14.90 | 667 |
| N,N-Di-tertbutylethylenediamine | 5 | 100 | 2.00 | 50 |
| | 5 | 178 | 3.56 | 50 |
| | 5 | 316 | 6.32 | 50 |
| | 5 | 562 | 11.24 | 50 |
| | 5 | 1000 | 1.22 | 820 |
| | 5 | 10,000 | 12.20 | 820 |

Appropriate amounts of the test materials were suspended in 0.5% sodium carboxymethylcellulose and administered to the rats in a single oral dose by oral intubation. Initial body weights ranged from 152 to 253 grams. The rats in all of the groups were observed for mortality and signs of toxic and pharmacologic effect immediately after dosing, at one and four hours postdosing, and once daily thereafter for a total of 14 days. Necropsies were performed on all animals which died during the study and on all animals sacrificed by chloroform overdoses at termination (Day 14). Mortality data were analyzed statistically by the method of Litchfield and Wilcoxon, J. Pharmacol and Exptl. Thera. 96, 99, 1949.

## RESULTS - DIIMINE

### Mortality

Cumulative mortality through Day 14 is presented in Table 1, p. 10. The acute oral $LD_{50}$ was calculated to be 1731.8 mg/kg of body weight with 95% confidence limits from 1418.7 to 2114.0 mg/kg.

### Clinical Signs

1000 mg/kg: Listlessness was noted from one hour postdose through Day 4 and rough fur was noted from 24 hours postdose through Day 2.

1470 mg/kg: One animal was found dead at four hours postdose. Antemortem signs noted in this animal and clinical signs noted in the surviving animals included listlessness, hyperpnea

and/or a hunched appearance, epistaxis, and rough fur from one hour postdose through Day 7 in one or more rats.

1780 mg/kg: Three of the animals were found dead at this level by Day 2. Antemortem signs noted in these animals and clinical signs noted in the surviving animals included listlessness, hyperpnea, a hunched appearance and/or ataxia, epistaxis, and rough fur from one hour postdose through Day 14 in one or more rats.

2150 mg/kg: All five of the animals died at this level. Antemortem signs included listlessness, hyperpnea, a hunched appearance and/or ataxia from one hour postdose through Day 4.

3160 mg/kg: All five of the animals died at this level. Antemortem signs included ataxia, listlessness, hyperpnea, a hunched appearance and muscular tremors.

4640 mg/kg and 10,000 mg/kg: All of the animals at both of these levels were found dead at one hour postdose.

Gross Pathology

Necropsy findings in the animals surviving the 14-day observation period included red spots on the lungs (2/5 animals at the 1000 mg/kg level); adhesions of the stomach to the spleen and abdominal wall, white and thickened stomach lining, and 1 cm x 1 cm orange area on the mucosa of the stomach (1/5 animals at the 1000 mg/kg level); sloughing, blanching and thickening of the stomach along with a yellowish-brown discoloration of the stomach (4/5 animals at the 1470 mg/kg level); and white, raised areas on

the stomach mucosa (3/5 animals at the 1780 mg/kg level). Necropsy findings in the animals found dead included dark red areas in the stomach and/or thickening, folding, or perforations of the stomachs of the animals at the 1470, 1780, 2150, 3160, and 4640 mg/kg levels. The dead animals at the 10,000 mg/kg level were noted to have thin and reddened gastrointestinal tracts.

## RESULTS - N,N-Dimethyl-N-Butylamine

### Mortality

Cumulative mortality through Day 14 is presented in Table 2 p. 11. The acute oral $LD_{50}$ was calculated to be 187.8 mg/kg of body weight with 95% confidence limits from 133.4 to 264.4 mg/kg.

### Clinical Signs

100 mg/kg: Listlessness, hyperpnea and a hunched appearance were noted from one hour postdose through Day 9. Rough fur was noted from Day 7 through Day 14.

178 mg/kg: Two animals were found dead by one hour postdose. Clinical signs noted in the surviving animals included listlessness, a hunched appearance, ataxia, rough fur, and muscular tremors from one hour postdose through Day 14.

316, 562, 1000, and 10,000 mg/kg: All of the animals at these levels were found dead within one hour postdose.

Gross Pathology

Necropsy findings in the animals surviving through the 14-day observation period were limited to thickening and blanching of the stomach (3/5 animals at the 178 mg/kg level) and adhesions of the stomach to the pancreas and abdominal wall (1/5 animals at the 178 mg/kg level). Necropsy findings in the animals found dead included extremely reddened livers and stomach linings, a leathery and blanched appearance of the stomach with an accumulation of red fluid, and/or perforations and reddening of the entire gastrointestinal tracts.

RESULTS - N,N-Dimethylethylamine

Mortality

Cumulative mortality through Day 14 is presented in Table 3, p. 12. The acute oral $LD_{50}$ was calculated to be 593.4 mg/kg of body weight with 95% confidence limits from 421.3 to 835.8 mg/kg.

Clinical Signs

100 mg/kg: Listlessness was noted from one hour postdose through Day 6 and rough fur was noted from Day 5 through Day 14.

178 mg/kg: Listlessness, a hunched appearance, rough fur, and/or excessive salivation were noted from one hour postdose through Day 14.

316 mg/kg: Listlessness, a hunched appearance and/or ataxia, hyperpnea, and rough fur were noted from one hour postdose through Day 14.

562 mg/kg: One animal was found dead within four hours postdose. Antemortem signs noted in this animal and clinical signs noted in the surviving animals include listlessness, hyperpnea, ataxia, a hunched appearance and/or excessive salivation, urine staining, epistaxis, chromodacryorrhea, and rough fur from one hour postdose through Day 14 in one or more rats.

1000 and 10,000 mg/kg: All five of the animals at the 1000 mg/kg level and four of the animals at the 10,000 mg/kg level were found dead at one hour postdose. The one animal alive at the 10,000 mg/kg level was prostrate at one hour postdose and was found dead at four hours postdose.

Gross Pathology

Necropsy findings in the animals surviving through the 14-day observation period included a thickened or leathery appearance and blanching of the stomach walls (5/5 animals at the 178 and 316 mg/kg levels) and deeply reddened gastrointestinal tracts with an accumulation of red fluid and deeply reddened kidneys (2/5 animals at the 562 mg/kg level). Necropsy findings in the animals found dead included a thickened or leathery appearance and blanching of the stomachs, multiple adhesions of the abdominal cavity and/or perforations and reddening of the entire gastrointestinal tracts and reddening of the kidneys.

RESULTS - N,N-Di-tertbutylethylenediamine

Mortality

Cumulative mortality through Day 14 is presented in Table 4, p. 13. The acute oral $LD_{50}$ was calculated to be 477.7 mg/kg of body weight with 95% confidence limits from 340.1 to 670.9 mg/kg.

Clinical Signs

100 mg/kg: Listlessness was noted from one hour postdose through 24 hours postdose.

178 mg/kg: Listlessness, a hunched appearance and/or ataxia, hyperpnea, and rough fur were noted from one hour postdose through Day 14.

316 mg/kg: Listlessness, a hunched appearance and rough fur were noted from one hour postdose through Day 14.

562 mg/kg: One of the animals was found dead at four hours postdose, two of the animals were found dead on Day 2 and one of the animals was found dead on Day 4. Antemortem signs noted in these animals and clinical signs noted in the surviving animal include a hunched appearance, hyperpnea, listlessness and/or ataxia, bloody salivation, epistaxis, prostration, muscle spasms, and rough fur from one hour postdose through Day 14.

1000 mg/kg: All five of the animals were dead within 24 hours postdose. Antemortem signs included listlessness, excessive salivation, hyperpnea, muscle spasms and/or gasping and prostration.

10,000 mg/kg: All five of the animals were dead within four hours postdose. Antemortem signs included listlessness, excessive salivation, hyperpnea, muscle spasms and/or prostration.

Gross Pathology

Necropsy findings in the animals surviving through the 14-day observation period were limited to blanching of the stomach walls (2/5 animals at the 178 and 316 mg/kg levels and 1/5 animals at the 562 mg/kg level) and thickened livers (5/5 animals at the 316 mg/kg level and 1/5 animals at the 562 mg/kg level). Necropsy findings in the animals found dead included reddened, toughened and perforated gastrointestinal tracts and/or reddened kidneys, accumulation of dark red fluid in the abdominal cavity, and a hard, solid, white crystalline material in the stomach.

Toxicity Classification

Because the $LD_{50}$ for all four chemicals is between 50 and 5000 mg/kg, these chemicals would be considered orally toxic. The degree of toxicity from least to most toxic, is Diimine, N,N-Dimethylethylamine, N,N-Di-tertbutylethylenediamine, and N,N-Dimethyl-N-Butylamine.

Submitted by Frederick E. Reno
FREDERICK E. RENO, Ph. D.
Director
Toxicology Department

Report Preparation: Andrews
Supervision: Kapp

mra

Table 1 - Cumulative Mortality[a]

Acute Oral Toxicity Study of Diimine in Rats

| Dose Level mg/kg | Time After Dosing | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Immediately | Hour | | | Day | | | |
| | | 1 | 4 | 24 | 2 | 3 | 4 | 5-14 |
| 1000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1470 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| 1780 | 0 | 1 | 1 | 2 | 2 | 2 | 2 | 2 |
| 2150 | 0 | 0 | 2 | 4 | 4 | 4 | 4 | 5 |
| 3160 | 0 | 4 | 4 | 5 | 5 | 5 | 5 | 5 |
| 4640 | 0 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 10,000 | 0 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |

[a]Number dead out of five rats/level.

$LD_{50}$ = 1731.8 mg/kg of body weight with 95% confidence limits from 1418.7 to 2114.0 mg/kg.

Table 2 - Cumulative Mortality[a]

Acute Oral Toxicity Study of N,N-Dimethyl-N-Butylamine in Rats

| Dose Level mg/kg | Time After Dosing | | | | |
|---|---|---|---|---|---|
| | Immediately | Hour | | | Day |
| | | 1 | 4 | 24 | 2 - 14 |
| 100 | 0 | 0 | 0 | 0 | 0 |
| 178 | 0 | 2 | 2 | 2 | 2 |
| 316 | 0 | 4 | 5 | 5 | 5 |
| 562 | 0 | 5 | 5 | 5 | 5 |
| 1000 | 0 | 5 | 5 | 5 | 5 |
| 10,000 | 0 | 5 | 5 | 5 | 5 |

[a]Number dead out of five rats/level.

$LD_{50}$ = 187.8 mg/kg of body weight with 95% confidence limits from 133.4 to 264.4 mg/kg.

Table 3 - Cumulative Mortality[a]

Acute Oral Toxicity Study of N,N-Dimethylethylamine in Rats

| Dose Level mg/kg | Time After Dosing | | | | |
|---|---|---|---|---|---|
| | Immediately | Hour | | | Day |
| | | 1 | 4 | 24 | 2 - 14 |
| 100 | 0 | 0 | 0 | 0 | 0 |
| 178 | 0 | 0 | 0 | 0 | 0 |
| 316 | 0 | 0 | 0 | 0 | 0 |
| 562 | 0 | 0 | 1 | 1 | 2 |
| 1000 | 0 | 5 | 5 | 5 | 5 |
| 10,000 | 0 | 4 | 5 | 5 | 5 |

[a]Number dead out of five rats/level.

$LD_{50}$ = 593.4 mg/kg of body weight with 95% confidence limits from 421.3 to 835.8 mg/kg.

Table 4 - Cumulative Mortality[a]

Acute Oral Toxicity Study of N,N-Di-tertbutylethylenediamine in Rats

| Dose Level mg/kg | Time After Dosing | | | | | | |
|---|---|---|---|---|---|---|---|
| | Immediately | Hour | | | Day | | |
| | | 1 | 4 | 24 | 2 | 3 | 4 - 14 |
| 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 178 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 316 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 562 | 0 | 0 | 1 | 1 | 3 | 3 | 4 |
| 1000 | 0 | 0 | 2 | 5 | 5 | 5 | 5 |
| 10,000 | 0 | 2 | 5 | 5 | 5 | 5 | 5 |

[a]Number dead out of five rats/level.

$LD_{50}$ = 477.7 mg/kg of body weight with 95% confidence limits from 340.1 to 670.9 mg/kg.

CONFIDENTIAL

# N,N-DIMETHYL-DIPROPYLENTRIAMIN:

# OECD 401 ACUTE ORAL TOXICITY

# TEST IN THE RAT

# PROJECT NUMBER 34/4

T01437

Experimental Procedures:

Date Started: 12 November 1985

Date Completed: 10 December 1985

AUTHORS: J.R. Jones

T.A. Collier

STUDY SPONSOR:

Ruhrchemie Aktiengesellschaft
Postfach 1301 60
D-4200 Oberhausen 11
WEST GERMANY

ISSUED BY:

Safepharm Laboratories Limited
P.O. Box No. 45
DERBY
DE1 2BT
U.K.

Telephone: DERBY (0332) 792789/
792896

Telex: 377079 SAFPHM G

QUALITY ASSURANCE UNIT

SAFEPHARM LABORATORIES LIMITED

## STATEMENT OF COMPLIANCE

This study was carried out in compliance with OECD Principles of Good Laboratory Practice.

The routine inspection of short term toxicity studies at Safepharm Laboratories is carried out as a continuous process designed to ensure that where possible all critical phases of a particular study type are inspected at least once per month.

This report has been audited by Safepharm Laboratories Quality Assurance Unit and is an accurate account of the procedures followed and accurately records the original raw laboratory data generated in this study.

M.B. Thomas B.Sc., M.I. Biol.

QUALITY ASSURANCE MANAGER .....

DATE: 17/12/85

# V A L I D A T I O N

We the undersigned hereby declare that this study was performed under our supervision according to the procedures herein described, and that this report provides an accurate and faithful record of the results obtained.

..... DATE: 17/12/85

J.R. JONES HNC

Study Director

..... DATE: 17/12/85

T.A. COLLIER HNC

Director of Toxicology

# SUMMARY OF RESULTS

STUDY SPONSOR : RUHRCHEMIE AKTIENGESELLSCHAFT

TEST MATERIAL : N,N-DIMETHYL-DIPROPYLENTRIAMIN

## ACUTE ORAL MEDIAN LETHAL DOSE ($LD_{50}$) IN THE RAT

| | | |
|---|---|---|
| ALL ANIMALS | : | 1710 mg/kg (1361 - 2149) |
| MALES ONLY | : | BEST ESTIMATE BETWEEN 1000 AND 2924 mg/kg |
| FEMALES ONLY | : | 1804 mg/kg (1387 - 2347) |

# N,N-DIMETHYL-DIPROPYLENTRIAMIN:
# OECD 401 ACUTE ORAL TOXICITY
# TEST IN THE RAT
# PROJECT NUMBER 34/4

## INTRODUCTION

The study was performed according to Safepharm Standard Protocol Number GM 04/82/44C and was designed to assess the acute oral toxicity of the test material in the rat. The study was designed to comply with the recommendations of the OECD Guidelines for Testing of Chemicals (1981) No. 401 "Acute Oral Toxicity". The test system was chosen because the rat has been shown to be a suitable model for this type of study and is recommended in the test method.

The results of the study are believed to be of value in predicting the likely toxicity of the test material to man.

## METHODS

1. Animals and Animal Husbandry

   Twenty-four male and twenty-four female Sprague-Dawley CFY strain rats were supplied by Interfauna U.K. Limited, Wyton, Huntingdon. At the start of the study the males weighed 106 - 142g, and the females 109 - 138g, and were approximately five to eight weeks old. After a minimum acclimatisation period of five days the animals were selected at random and given a unique number within the study by ear punching and a number written on a colour coded cage card.

   The animals were housed in groups of up to five by sex in polypropylene cages with sawdust bedding. With the exception of an overnight fast immediately before dosing and for approximately two hours after dosing, free access to mains drinking water and food (Rat and Mouse Expanded Diet No.1, Special Diet Services Limited, Witham, Essex, U.K.) was allowed throughout the study.

1. Animals and Animal Husbandry (contd)

The animal room was maintained at a temperature of 18 - 23°C and relative humidity of 45 - 65%. The rate of air exchange was approximately 15 changes per hour and the lighting was controlled by a time switch to give 12 hours light and 12 hours darkness.

2. Test Material and Experimental Preparation

The test material was supplied by Ruhrchemie Aktiengesellschaft as follows:

| | | |
|---|---|---|
| Description | : | Clear, colourless liquid |
| Container | : | Glass screw-top bottle |
| Label | : | N,N-Dimethylpropylentriamin |
| Batch number | : | 0407102 |
| Date of arrival | : | 22 October 1985 |
| Storage conditions | : | Room temperature |

For the purpose of this study the test material was used as supplied. The specific gravity was used to calculate the required dose volumes for the appropriate dose levels.

The identification and stability of the test material were not determined.

3. Procedure

a) Range-finding Study

A preliminary study was performed using groups of four rats (two males and two females) to determine suitable dose levels for the main study.

| NUMBER OF RATS MALE | FEMALE | DOSE LEVEL mg/kg | SPECIFIC GRAVITY | DOSE VOLUME ml/kg |
|---|---|---|---|---|
| 2 | 2 | 1000 | 0.87 | 1.15 |
| 2 | 2 | 5000 | 0.87 | 5.75 |

3. Procedure

a) Range-finding Study (contd)

Animals were observed 1 and 4 hours after dosing and then daily for five days, or until all evidence of toxicity had subsided, whichever was the longer.

Individual bodyweights were recorded on the day of dosing to allow the calculation of individual treatment volumes. No necropsies were performed.

b) Main Study

Four groups of ten rats (five males and five females) were dosed at logarithmically spaced dose levels, selected using the results of the range-finding study as follows:

| NUMBER OF RATS MALE | FEMALE | DOSE LEVEL mg/kg | SPECIFIC GRAVITY | DOSE VOLUME ml/kg |
|---|---|---|---|---|
| 5 | 5 | 1000 | 0.87 | 1.15 |
| 5 | 5 | 1710 | 0.87 | 1.97 |
| 5 | 5 | 2924 | 0.87 | 3.36 |
| 5 | 5 | 5000 | 0.87 | 5.75 |

All animals were dosed once only at the appropriate dose level by gavage using a metal cannula attached to a graduated syringe. The dose administered to each animal was calculated according to its fasted bodyweight at the time of dosing.

Animals were observed 1 and 4 hours after dosing and subsequently once daily for 14 days. Mortalities and evidence of overt toxicity were recorded at each observation.

b) Main Study (contd)

Individual bodyweights were recorded on the day of treatment (day 0), days 7 and 14, and at death.

All animals were subjected to gross necropsy examination for any macroscopic abnormalities. No tissues were retained.

4. Evaluation of Data

Using the mortality data obtained the acute oral median lethal dose ($LD_{50}$) and 95% confidence limits of the test material were calculated using the method of Weil C.S. (1952) Biometrics 8,249. A separate $LD_{50}$ value and 95% confidence limits was calculated for females. A best estimate of the oral toxicity in males is presented.

## ARCHIVES

On completion of the study all raw data and a copy of the final report were transferred to Safepharm Laboratories Central Archives, London Road, Shardlow, Derbyshire, U.K.

## RESULTS

1. Range-finding Study

The mortalities are summarised as follows:

| DOSE LEVEL mg/kg | DEATHS ON DAY 0 | 1 | 2 | 3 | 4 | 5 | TOTAL DEATHS |
|---|---|---|---|---|---|---|---|
| 1000 | 0 | 0 | 0 | 0 | 0 | 0 | 0/4 |
| 5000 | 3 | 1 | - | - | - | - | 4/4 |

- = All animals dead

The mortalities indicated an oral $LD_{50}$ in the range 1000 - 5000 mg/kg.

2. Main Study

a) Mortalities

The mortalities are summarised as follows:

| DOSE LEVEL mg/kg | SEX | DEATHS ON DAY 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8-14 | TOTAL DEATHS |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1000 | MALE | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1/10 |
| | FEMALE | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 1710 | MALE | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 4/10 |
| | FEMALE | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 2924 | MALE | 5 | - | - | - | - | - | - | - | - | 10/10 |
| | FEMALE | 3 | 2 | - | - | - | - | - | - | - | |
| 5000 | MALE | 5 | - | - | - | - | - | - | - | - | 10/10 |
| | FEMALE | 5 | - | - | - | - | - | - | - | - | |

- = All animals dead

a) Mortalities (contd)

Using the mortality data the acute oral median lethal dose ($LD_{50}$) and 95% confidence limits of the test material were calculated by the prescribed statistical method to be:

| | | |
|---|---|---|
| All animals | : | 1710 mg/kg (1361 - 2149) |
| Males only | : | Best estimate between 1000 and 2924 mg/kg |
| Females only | : | 1804 mg/kg (1387 - 2347) |

All high dose animals were found dead four hours after treatment; three males and two females treated with 2924 mg/kg and one female treated with 1710 mg/kg were also found dead at this time. Two males and one female treated with 2924 mg/kg and one female treated with 1710 mg/kg were found dead at the afternoon death check approximately seven hours after treatment. All other deaths were noted on days 1-2 after treatment.

b) Clinical Observations

Clinical observations are given in Table 1.

All animals treated with 1000 mg/kg showed hunched posture and pilo-erection 1 and 4 hours after treatment. Isolated or occasional signs of toxicity noted during this period were lethargy, decreased respiratory rate or ptosis. All surviving animals continued to show hunched posture, pilo-erection, lethargy and decreased respiratory rate on day 1 with isolated instances of ptosis and diarrhoea. All surviving animals recovered and appeared normal 3-4 days after treatment.

All animals treated with 1710 mg/kg also showed hunched posture and pilo-erection with occasional signs of lethargy and decreased respiratory rate 1 and 4 hours after treatment. Surviving animals continued to show

b) Clinical Observations (contd)

these signs of toxicity up to 3 days after treatment. Occasional or isolated signs of toxicity noted were ptosis, ataxia, diarrhoea, gasping respiration, loss of righting reflex, diuresis, red urine, pallor of the extremities and red/brown staining of the snout. All surviving animals were normal on day 4.

Animals treated with 2924 and 5000 mg/kg showed hunched posture, pilo-erection, lethargy, decreased respiratory rate and ataxia one hour after treatment. These signs continued to be noted in surviving animals treated with 2924 mg/kg at the 4-hour observation. There were no survivors from these dose groups.

c) Bodyweights

Individual bodyweights together with weekly bodyweight increases are given in Tables 2 and 3.

All surviving animals showed bodyweight gains throughout the study period.

d) Necropsy

Individual necropsy findings are given in Table 4.

Common findings noted at necropsy of animals dying during the study and at termination of the study were associated with the stomach, small intestine and livers. The non-glandular and glandular regions of the stomach were haemorrhaged with white raised areas or white thickening of the border between the two regions. The small intestine was also haemorrhaged, particularly in high dose groups. The livers were abnormal in appearance and were described as mottled. Occasional findings of congested lungs and dark spleens were also noted.

## CONCLUSION

The acute oral median lethal dose ($LD_{50}$) and 95% confidence limits of N,N-Dimethyl-dipropylentriamin in the rat was found to be:

| | | |
|---|---|---|
| All animals | : | 1710 mg/kg (1361 - 2149) |
| Males only | : | Best estimate between 1000 and 2924 mg/kg |
| Females only | : | 1804 mg/kg (1387 - 2347) |

## TABLE 1

TEST MATERIAL: N,N-DIMETHYL DIPROPYLENTRIAMIN

### CLINICAL OBSERVATIONS AND MORTALITIES

PROJECT NUMBER: 34/4

| DOSE LEVEL mg/kg | CLINICAL OBSERVATIONS | NUMBER SHOWING EFFECTS DURING DAY OF DOSING (HOUR) | | NUMBER SHOWING EFFECTS DURING DAY OF OBSERVATION | | | | | | | | TOTAL DEATHS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 4 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8-14 | |
| 1000 | Hunched posture | 10 | 10 | 9 | 9 | 4 | 0 | 0 | 0 | 0 | 0 | 1/10 |
| | Pilo-erection | 10 | 10 | 9 | 9 | 4 | 0 | 0 | 0 | 0 | 0 | |
| | Lethargy | 1 | 2 | 9 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Decreased respiratory rate | 0 | 1 | 9 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | |
| | Ptosis | 0 | 1 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Diarrhoea | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Normal | 0 | 0 | 0 | 0 | 5 | 9 | 9 | 9 | 9 | 9 | |
| | Death | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 1710 | Hunched posture | 10 | 9 | 7 | 6 | 6 | 0 | 0 | 0 | 0 | 0 | 4/10 |
| | Pilo-erection | 10 | 9 | 7 | 6 | 6 | 0 | 0 | 0 | 0 | 0 | |
| | Lethargy | 4 | 2 | 7 | 6 | 1 | 0 | 0 | 0 | 0 | 0 | |
| | Decreased respiratory rate | 2 | 2 | 7 | 6 | 6 | 0 | 0 | 0 | 0 | 0 | |
| | Ptosis | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Ataxia | 0 | 0 | 3 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | |
| | Diarrhoea | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Gasping respiration | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Loss of righting reflex | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Diuresis | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Red urine | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Pallor of the extremities | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Red/brown staining around snout | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Normal | 0 | 0 | 0 | 0 | 0 | 6 | 6 | 6 | 6 | 6 | |
| | Death | 0 | 2* | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | |

* = one animal found dead at afternoon death check approximately 7 hours after treatment

TABLE 1 (contd)

TEST MATERIAL: N,N-DIMETHYL-DIPROPYLENTRIAMIN

CLINICAL OBSERVATIONS AND MORTALITIES

PROJECT NUMBER: 34/4

| DOSE LEVEL mg/kg | CLINICAL OBSERVATIONS | NUMBER SHOWING EFFECTS DURING DAY OF DOSING (HOUR) | | NUMBER SHOWING EFFECTS DURING DAY OF OBSERVATION | | | | | | | | TOTAL DEATHS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 4 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8-14 | |
| 2924 | Hunched posture | 10 | 5 | - | - | - | - | - | - | - | - | 10/10 |
| | Pilo-erection | 10 | 5 | - | - | - | - | - | - | - | - | |
| | Lethargy | 10 | 5 | - | - | - | - | - | - | - | - | |
| | Decreased respiratory rate | 10 | 5 | - | - | - | - | - | - | - | - | |
| | Ptosis | 5 | 4 | - | - | - | - | - | - | - | - | |
| | Ataxia | 3 | 5 | - | - | - | - | - | - | - | - | |
| | Death | 0 | 8* | 2 | - | - | - | - | - | - | - | |
| 5000 | Hunched posture | 10 | - | - | - | - | - | - | - | - | - | 10/10 |
| | Pilo-erection | 10 | - | - | - | - | - | - | - | - | - | |
| | Lethargy | 10 | - | - | - | - | - | - | - | - | - | |
| | Decreased respiratory rate | 10 | - | - | - | - | - | - | - | - | - | |
| | Ptosis | 10 | - | - | - | - | - | - | - | - | - | |
| | Ataxia | 10 | - | - | - | - | - | - | - | - | - | |
| | Death | 0 | 10 | - | - | - | - | - | - | - | - | |

* = two males and one female found dead at afternoon death check approximately 7 hours after treatment

\- = all animals dead

TABLE 2

INDIVIDUAL BODYWEIGHTS - MALES

TEST MATERIAL: N,N-DIMETHYL-DIPROPYLENTRIAMIN PROJECT NUMBER: 34/4

| DOSE LEVEL mg/kg | ANIMAL NUMBER | | BODYWEIGHT (g) ON DAY: 0 | 7 | 14 | AT DEATH | INCREMENT DURING WEEK 1 | 2 |
|---|---|---|---|---|---|---|---|---|
| 1000 | 5-0 | M | 113 | 160 | 194 | | 47 | 34 |
| | 5-1 | M | 122 | - | - | 107 | - | - |
| | 5-2 | M | 117 | 177 | 248 | | 60 | 71 |
| | 5-3 | M | 125 | 143 | 196 | | 18 | 53 |
| | 5-4 | M | 106 | 172 | 251 | | 66 | 79 |
| 1710 | 7-0 | M | 124 | 183 | 262 | | 59 | 79 |
| | 7-1 | M | 138 | 160 | 232 | | 22 | 72 |
| | 7-2 | M | 114 | - | - | 87 | - | - |
| | 7-3 | M | 120 | 177 | 249 | | 57 | 72 |
| | 7-4 | M | 121 | - | - | 76 | - | - |
| 2924 | 9-0 | M | 139 | - | - | 134 | - | - |
| | 9-1 | M | 132 | - | - | 127 | - | - |
| | 9-2 | M | 142 | - | - | 138 | - | - |
| | 9-3 | M | 141 | - | - | 137 | - | - |
| | 9-4 | M | 124 | - | - | 120 | - | - |
| 5000 | 11-0 | M | 131 | - | - | 128 | - | - |
| | 11-1 | M | 126 | - | - | 123 | - | - |
| | 11-2 | M | 132 | - | - | 125 | - | - |
| | 11-3 | M | 139 | - | - | 137 | - | - |
| | 11-4 | M | 120 | - | - | 116 | - | - |

- = animal dead

TABLE 3

INDIVIDUAL BODYWEIGHTS - FEMALES

TEST MATERIAL: N,N-DIMETHYL-DIPROPYLENTRIAMIN PROJECT NUMBER: 34/4

| DOSE LEVEL mg/kg | ANIMAL NUMBER | | BODYWEIGHT (g) ON DAY: 0 | 7 | 14 | AT DEATH | INCREMENT DURING WEEK 1 | 2 |
|---|---|---|---|---|---|---|---|---|
| 1000 | 6-0 | F | 134 | 172 | 214 | | 38 | 42 |
| | 6-1 | F | 132 | 158 | 188 | | 26 | 30 |
| | 6-2 | F | 127 | 180 | 226 | | 53 | 46 |
| | 6-3 | F | 138 | 186 | 224 | | 48 | 38 |
| | 6-4 | F | 129 | 179 | 214 | | 50 | 35 |
| 1710 | 8-0 | F | 130 | 174 | 213 | | 44 | 39 |
| | 8-1 | F | 134 | 173 | 206 | | 39 | 33 |
| | 8-2 | F | 129 | - | - | 124 | - | - |
| | 8-3 | F | 126 | - | - | 119 | - | - |
| | 8-4 | F | 111 | 130 | 183 | | 19 | 53 |
| 2924 | 10-0 | F | 112 | - | - | 108 | - | - |
| | 10-1 | F | 121 | - | - | 117 | - | - |
| | 10-2 | F | 137 | - | - | 123 | - | - |
| | 10-3 | F | 109 | - | - | 104 | - | - |
| | 10-4 | F | 132 | - | - | 126 | - | - |
| 5000 | 12-1 | F | 119 | - | - | 115 | - | - |
| | 12-2 | F | 116 | - | - | 111 | - | - |
| | 12-3 | F | 125 | - | - | 120 | - | - |
| | 12-4 | F | 121 | - | - | 117 | - | - |
| | 12-5 | F | 119 | - | - | 115 | - | - |

- = animal dead

TABLE 4

## INDIVIDUAL NECROPSY FINDINGS

TEST MATERIAL: N,N-DIMETHYL-DIPROPYLENTRIAMIN

PROJECT NUMBER : 34/4

| DOSE LEVEL mg/kg | MACROSCOPIC OBSERVATIONS | ANIMAL NUMBER AND SEX | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 5-0 | 5-1* | 5-2 | 5-3 | 5-4 | 6-0 | 6-1 | 6-2 | 6-3 | 6-4 |
| | | M | M | M | M | M | F | F | F | F | F |
| 1000 | Lungs : congested | | | | | | | P | | | |
| | Glandular region of stomach : haemorrhage | | P | | | | | | | | |
| | : white thickening on border with non-glandular region | | | | P | | | | | | |
| | Non-glandular region of stomach : haemorrhage | | P | | | | | | | | |
| | : white raised area | | | | P | P | | P | | P | |
| | No abnormalities detected (✓) | ✓ | | ✓ | | | ✓ | | ✓ | | ✓ |
| | | 7-0 | 7-1 | 7-2* | 7-3 | 7-4* | 8-0 | 8-1 | 8-2* | 8-3* | 8-4 |
| | | M | M | M | M | M | F | F | F | F | F |
| 1710 | Glandular region of stomach : haemorrhage | | | | | | | | P | P | |
| | : white thickening on border with non-glandular region | | | | P | | P | P | | | P |
| | Non-glandular region of stomach : haemorrhage | | | | | | | | | P | |
| | : white raised areas | P | P | | P | | P | P | | | P |
| | Small intestine : haemorrhage | | | | | | | | P | P | |
| | Animal cannibalised - Necropsy not performed (NP) | | | NP | | NP | | | | | |
| | | 9-0* | 9-1* | 9-2* | 9-3* | 9-4* | 10-0* | 10-1* | 10-2* | 10-3* | 10-4* |
| | | M | M | M | M | M | F | F | F | F | F |
| 2924 | Lungs : congested | P | | | P | P | P | | P | | P |
| | Liver : mottled | P | P | P | P | P | P | P | P | P | P |
| | Glandular region of stomach : haemorrhage | P | P | P | P | P | P | | P | P | P |
| | Non-glandular region of stomach : haemorrhage | P | P | P | P | P | P | P | P | P | P |
| | Small intestine : haemorrhage | | | | | | | | | | |
| | | 11-0* | 11-1* | 11-2* | 11-3* | 11-4* | 12-0* | 12-1* | 12-2* | 12-3* | 12-4* |
| | | M | M | M | M | M | F | F | F | F | F |
| 5000 | Liver : mottled | | | P | | | | P | | | P |
| | Spleen : dark | | P | | | | | | | | P |
| | Glandular region of stomach : haemorrhage | P | P | P | P | P | P | P | P | P | P |
| | Non-glandular region of stomach : haemorrhage | P | P | P | P | P | P | P | P | P | P |
| | Small intestine : haemorrhage | P | P | P | P | P | P | P | P | P | P |

* = animal died during study

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# Isononylamin

## Testing for acute oral toxicity in the male and female Wistar rat

| | |
|---|---|
| **Author:** | Dr. U. Jensch |
| **Report number:** | 97.0059 |
| **Date of report:** | February 03, 1997 |
| **Study number:** | 96.0848 |
| **Origin of report:** | Hoechst Marion Roussel<br>Preclinical Development Germany<br>Drug Safety<br>65926 Frankfurt am Main<br>GERMANY |

This report contains 32 pages

T 787

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# 1 SUMMARY / ABSTRACT

The acute oral toxicity testing of Isononylamin in the Wistar rat yielded the following median lethal dose (LD50):

Male animals: 315 - 500 mg/kg body weight

Female animals did not show a higher sensitivity to the test substance.

Lethality occurred up to day two of the study. Beside unspecific symptoms the animals showed impairments of motility and respiration and narrowed palpebral fissure. Additionally, stupor, coat bristling, prone position, diarrhea, straddling hind limps, pale skin, trembling as well as tonoclonic and saltatory and rolling convulsions were observed.
*4 days after application all clinical signs were reversible.*

Development of body weight was not impaired.

Necropsy of the decedent animals revealed light discoloured liver, light discoloured spleen, red and orange discoloured lungs, diffuse reddening and detachment of the stomach mucosa, small intestine full of yellowish mucous and reddish-black mass, Haemoccult positive, diffuse reddening and detachment of the small intestine mucosa.

The animals killed at the end of the observation period showed no macroscopically visible changes.

# 2 STATEMENT

This report contains the unpublished research findings of Hoechst scientists. It should not be published, in whole or in part, or referred to in any publication without authorization from the company.

## 2.1 GLP Compliance Statement

This study was conducted in compliance with Good Laboratory Practice regulations. No unforeseen circumstances were observed which might have affected the quality or integrity of the study.

Study Director: _____ February 03, 1997
(Dr. U. Jensch)

Testing Facility Management: _____ Feb 4, 1997
(Dr. H. H. Donaubauer)

Quality Assurance (GLP)

03.02.1997

Quality Assurance Statement

Title: Isononylamin
Testing for acute oral toxicity
in the male and female Wistar rat

Study: 96.0848

This study was periodically inspected and properly signed records of these inspections were submitted to testing facility management and the study director as shown below:

| Inspection | Report |
|---|---|
| 10.12.1996 | 10.12.1996 |
| 19.12.1996 | 19.12.1996 |
| 03.02.1997 | 03.02.1997 |
| 03.02.1997 | 03.02.1997 |

03.02.1997

U. Müller

Quality Assurance (GLP)

# 3 OBJECTIVE / GUIDELINES

## 3.1 Objective

Testing for acute oral toxicity is an initial step in identifying the toxicological properties of a substance. It provides information on health risks resulting from a single oral intake and serves as a basis for classification. It permits the selection of optimum dose levels for repeated administration of the test substance. The Wistar rat has proved to be a suitable species for acute oral toxicity testing with many different substances.

## 3.2 Guidelines

The present study was conducted in compliance with

**EEC-Guideline B.1 "Acute Oral Toxicity" of the Directive 92/69/EEC; Commission Directive of July 31, 1992 adapting to technical progress for the seventeenth time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances**

and

**OECD Guidelines for Testing of Chemicals, 401 „Acute Oral Toxicity"; OECD 1987, updated Guideline, adopted: February 24, 1987**

This study was conducted in compliance with the

**Principles of Good Laboratory Practice, annex of paragraph 19a, section 1 of the chemical law of July 25, 1994**

# 4 SURVEY

| | | |
|---|---|---|
| Type of Study | : | Acute oral toxicity |
| Study Number | : | 96.0848 |
| Artemis Number | : | AR1171 |
| Test compound | : | Isononylamin |
| Sponsor | : | Division A, Factory Ruhrchemie, Section SU Umwelt |
| Test system | : | Wistar rat / male and female |
| Start of study | : | December 11, 1996 |
| End of study | : | January 22, 1997 |
| Dose levels male animals | : | 315, 400 and 500 mg/kg body weight |
| female animals | : | 400 mg/kg body weight |

## 4.1 Responsibilities

| | | |
|---|---|---|
| Study director | : | Dr. U. Jensch |
| Testing facility management | : | Dr. H. H. Donaubauer |
| Analytical Toxicology | : | Dr. H.-J. Pletsch |
| Quality Assurance (GLP) | : | Ap. S. Harston |
| Testing facility and archive | : | Hoechst Marion Roussel<br>Preclinical Development Germany<br>Drug Safety<br>65926 Frankfurt am Main<br>GERMANY |

# 5 MATERIAL AND METHODS

## 5.1 Test compound

| | | |
|---|---|---|
| Name | : | Isononylamin |
| Synonyms | : | mixture of Nonylamine |
| Chemical name | : | 3.5.5-Trimethyl-hexylamin (ca. 90%) |
| CAS-Number | : | 27775-00-4 |
| Product number / Code | : | 15JRNN005 |
| Molecular formula | : | $C_9H_{21}N$ |
| Molecular weight | : | 143.27 g/mol |
| Specific gravity | : | 0.786 kg/l |
| pH value | : | 11.6 |
| Certificate of analysis | : | November 06, 1996 |
| Content | : | Kohlenwasserstoffe + 0.02 %<br>Vorlaufkomponenten:<br>Nonylamine / Vorlaufisomere 3.93 %<br>3.5.5-Trimethylhexylamine 91.67 %<br>Nonylamine / Nachlaufisomere 4.31 %<br>Nonylalkohole 0.03 %<br>Schiff'sche Base 0.01 %<br>Wasser 0.03 % |
| Appearance | : | clear fluid |
| Melting point | : | < -70°C |
| Boiling point | : | 177°C by 1013 hPa |
| Solubility | : | 1-10 g/l in water<br>> 10 g/l in PEG, Ethanol and Acetone |
| Batch number | : | B 7516 |
| Date of production | : | September 1996 |
| Date of receiving | : | November 15, 1996 |
| Date of expiry | : | December 1997 |
| Storage conditions | : | darkness at room temperature in a fume cupboard |
| Stability and homogeneity in the vehicle | : | is guaranteed for 4 hours |

## 5.2 Test system

| | | |
|---|---|---|
| Species | : | Wistar rat |
| Strain | : | Hoe: WISKf(SPF71) |
| Origin | : | Hoechst Aktiengesellschaft, Kastengrund, SPF breeding colony |
| Body weight at start of study | | |
| male animals | : | mean = 182 g (= 100 %)<br>s = ± 7.8 g<br>min = 171 g (- 6.0 %)<br>max = 197 g (+ 8.2 %)<br>n = 15 |
| female animals | : | mean = 162 g (= 100 %)<br>s = ± 2.4 g<br>min = 159 g (- 1.9 %)<br>max = 165 g (+ 1.9 %)<br>n = 5 |
| Age at the start of the study | : | male animals approximately 7 weeks<br>female animals approximately 8 weeks |
| Randomization | : | Randomization schemes 96.0603, 96.0602, 96.0495 |
| Animal maintenance | : | in fully air-conditioned rooms in macrolon cages (type 4) on soft wood granulate in groups of 5 animals |
| Room temperature | : | 22 ± 3°C |
| Relative humidity | : | 50 ± 20 % |
| Lighting time | : | 12 hours daily |
| Acclimatization | : | at least one day<br>(breeding at identical conditions) |
| Food | : | ssniff® R/M-H (V 1534), ad *libitum* |
| Withdrawal of food | : | from about 16 hours before to 3 - 4 hours after treatment |
| Water | : | tap water in plastic bottles, ad libitum |
| Animal identification | : | fur marking with $KMnO_4$ and cage numbering |

## 5.3 Test groups

Based on a dose range finding study the following dose levels of Isononylamin were tested:

| Dose mg/kg b.w. | Concentration in % (w/v) | Volume applied (ml/kg body weight) | Number of males | Number of females |
|---|---|---|---|---|
| 315 | 3.15 | 10 | 5 | - |
| 400 | 4.00 | 10 | 5 | 5 |
| 500 | 5.00 | 10 | 5 | - |

## 5.4 Preparation of the test compound

Isononylamin was dissolved in the stated concentrations in sesame oil (Oleum sesami Ph. Eur. III, Fa. Mainland Pharmazeutische Fabrik GmbH) and distributed homogeneously by means of a magnetic stirrer.

The stability and the homogeneity of the test substance in the vehicle was determined by analytical methods.

## 5.5 Test procedure

The test substance preparations were administered by gavage to fasted animals at the stated dosage. The observation period following treatment lasted for 14 days. Symptoms and lethality were recorded twice every day (in the morning and in the afternoon), on weekends and public holidays only once. Animals found dead were dissected as soon as possible and examined for macroscopically visible changes. During this time the animals were weighed weekly. At the end of the observation period surviving animals were killed by carbon dioxide asphyxiation, dissected and also examined for macroscopically visible changes.

# 6 RESULTS

## 6.1 Lethality

Under the conditions described above, the following lethality rates were observed:

| Dose mg/kg b.w. | Lethality male animals absolute | male animals relative (%) | female animals absolute | female animals relative (%) |
|---|---|---|---|---|
| 315 | 0 | 0 | - | - |
| 400 | 3 | 60 | 2 | 40 |
| 500 | 5 | 100 | - | - |
| LD50 (mg/kg b.w.) | 315 - 500 | | - | |

## 6.2 Body weight development and clinical signs

Development of body weight was not impaired.

The following clinical signs were observed after the application of Isononylamin: decreased spontaneous activity, squatting posture, stilted, uncoordinated and ataxic gait, prone position, flanks drawn in, coat bristling, narrowed palpebral fissure, irregular respiration, respiration sounds, painting, increased respiration rate, stupor, diarrehea, straddling hind limps, pale skin, trembling, tonoclonic as well as saltatory and rolling convulsions.

(for individual data on clinical signs and development of body weight, see Appendix)

## 6.3 Autopsy findings

Macroscopic examination of the animals found dead revealed the following findings:

- Liver - light discoloration, unequal surface
- Spleen - light discoloration
- Lungs - discolored red and orange
- Stomach - diffuse reddening and detechment of the mucosa
- Small intestine - full of yellowish mucous, full of reddish-black mass, Haemoccult positive, diffuse reddening, detechment of the mucosa

The animals killed at the end of the observation period showed no macroscopically visible changes.

(for individual autopsy findings, see Appendix)

## 7 CONCLUSION

Based on the results obtained in this study the median lethal dose value ($LD_{50}$) of Isononylamin for the male Wistar rat lies between 315 - 500 mg/kg body weight.

Dr. UEJ / LW

audited by Quality Assurance (GLP)
08.02.1997

Hoechst Marion Roussel
Preclinical Development Germany
Drug Safety

Feburary 03, 1997
Dr. U. Jensch
Study Director

Feb 4, 1997
Dr. H. H. Donaubauer
Testing Facility Management

# 8 APPENDIX

**Dose range finding study**

Test substance : Isononylamin
Vehicle : Sesame oil
Concentration : 20 % / 2 %
Test species / sex : Wistar rat / male and female

| dose mg/kg b.w. | sex (m/f) | date of application | body weight days after application 0 | 7 | 14 | lethality time p.a. | b.w. [g] |
|---|---|---|---|---|---|---|---|
| 2000 | m | 11.12.96 | 170 | - | - | 35' | - |
| | f | 11.12.96 | 160 | - | - | 30' | - |
| 1000 | m | 11.12.96 | 168 | - | - | 45' | - |
| | f | 11.12.96 | 159 | - | - | 45' | - |
| 500 | m | 11.12.96 | 168 | - | - | 4h10' | - |
| | f | 11.12.96 | 157 | - | - | 3h30' | - |
| 200 | m | 16.12.96 | 179 | 256 | 306 | - | - |
| | f | 16.12.96 | 172 | 200 | 227 | - | - |
| 25 | m | 16.12.96 | 179 | 236 | 276 | - | - |
| | f | 16.12.96 | 173 | 213 | 232 | - | - |

**Comprehensive description of clinical signs:**

After application of 2000, 1000 and 500 mg/kg body weight the animals showed beside unspecific symptoms impairments of motility and respiration and narrowed palpebral fissure. Additionally,increased salivation, stupor, coat bristling, prone position, pale skin, trembling as well as tonoclonic and saltatory and rolling convulsions.

After application of 200 and 25 mg/kg body weight the animals showed impairments of motility and respiration.

**Comprehensive description of macroscopic findings:**

Macroscopic examination of the animals found dead revealed the following findings:

- Liver - light discoloration
- Spleen - light discoloration
- Lungs - discolored orange
- Stomach - diffuse reddening
- Small intestine - full of reddish-black mass, Haemoccult positive, diffuse reddening,

The animals killed at the end of the observation period showed no macroscopically visible changes.

LAC910-09/04 LAC910P/2 RUN DATE: 22/01/97

HOECHST MARION ROUSSEL SECTION: PRECL.DEV.GERMANY, DRUG SAFETY

INDIVIDUAL BODY WEIGHT DATA (g) PAGE: 2

STUDY NO: AR1171 TITLE: STUDY NO. 96.0848 ORAL ADMINISTRATION

GROUP: 2 COMPOUND: A00063/001

DOSE: 315 MG/KG

| ANIMAL NUMBER | DAY 1 | DAY 2 | DAY 3 | DAY 4 | DAY 5 | DAY 6 | DAY 7 | DAY 8 | DAY 9 | DAY 10 | DAY 11 | DAY 12 | DAY 13 | DAY 14 | DAY 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MALES | | | | | | | | | | | | | | | |
| 11 | 177 | - | - | - | - | - | - | 219 | - | - | - | - | - | - | 292 (D) |
| 12 | 181 | - | - | - | - | - | - | 236 | - | - | - | - | - | - | 265 (D) |
| 13 | 175 | - | - | - | - | - | - | 231 | - | - | - | - | - | - | 277 (D) |
| 14 | 184 | - | - | - | - | - | - | 233 | - | - | - | - | - | - | 256 (D) |
| 15 | 173 | - | - | - | - | - | - | 230 | - | - | - | - | - | - | 269 (D) |
| MEAN | 178.0 | | | | | | | 229.8 | | | | | | | 271.8 |
| S.D. | 4.5 | | | | | | | 6.5 | | | | | | | 13.6 |

(D) ANIMAL DEAD

LAC910-09/04 LAC910P/2 RUN DATE: 22/01/97

HOECHST MARION ROUSSEL SECTION: PRECL.DEV.GERMANY, DRUG SAFETY

INDIVIDUAL BODY WEIGHT DATA (g) PAGE: 1

STUDY NO: AR1171 TITLE: STUDY NO. 96.0848 ORAL ADMINISTRATION

GROUP: 1 COMPOUND: A00063/001

DOSE: 400 MG/KG

| ANIMAL NUMBER | DAY 1 | DAY 2 | DAY 3 | DAY 4 | DAY 5 | DAY 6 | DAY 7 | DAY 8 | DAY 9 | DAY 10 | DAY 11 | DAY 12 | DAY 13 | DAY 14 | DAY 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MALES | | | | | | | | | | | | | | | |
| 1 | 179 | - | - | - | - | - | - | 230 | - | - | - | | | - | 279 (D) |
| 2 | 186 (D) | | | | | | | | | | | | | | |
| 3 | 197 | - | - | - | - | - | - | 248 | - | - | - | - | | - | 322 (D) |
| 4 | 196 (D) | | | | | | | | | | | | | | |
| 5 | 177 (D) | | | | | | | | | | | | | | |
| MEAN | 187.0 | | | | | | | 239.0 | | | | | | | 300.5 |
| S.D. | 9.3 | | | | | | | 12.7 | | | | | | | 30.4 |
| FEMALES | | | | | | | | | | | | | | | |
| 6 | 160 (D) | | | | | | | | | | | | | | |
| 7 | 165 (D) | | | | | | | | | | | | | | |
| 8 | 163 | - | - | - | - | - | - | 199 | - | - | - | - | | - | 229 (D) |
| 9 | 159 | - | - | - | - | - | - | 152 | - | - | - | - | | - | 182 (D) |
| 10 | 163 | - | - | - | - | - | - | 196 | - | - | - | - | | - | 219 (D) |
| MEAN | 162.0 | | | | | | | 182.3 | | | | | | | 210.0 |
| S.D. | 2.4 | | | | | | | 26.3 | | | | | | | 24.8 |

(D) - ANIMAL DEAD

LAC910-09/04 LAC910P/2 RUN DATE: 22/01/97

HOECHST MARION ROUSSEL SECTION: PRECL.DEV.GERMANY, DRUG SAFETY

INDIVIDUAL BODY WEIGHT DATA (g) PAGE: 3

STUDY NO: AR1171 TITLE: STUDY NO. 96.0848 ORAL ADMINISTRATION

GROUP: 3 COMPOUND: A00063/001

DOSE: 500 MG/KG

| ANIMAL NUMBER | DAY 1 | DAY 2 | DAY 3 | DAY 4 | DAY 5 | DAY 6 | DAY 7 | DAY 8 | DAY 9 | DAY 10 | DAY 11 | DAY 12 | DAY 13 | DAY 14 | DAY 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MALES | | | | | | | | | | | | | | | |
| 16 | 184 (D) | | | | | | | | | | | | | | |
| 17 | 171 (D) | | | | | | | | | | | | | | |
| 18 | 176 | 164 (D) | | | | | | | | | | | | | |
| 19 | 188 | 179 (D) | | | | | | | | | | | | | |
| 20 | 187 (D) | | | | | | | | | | | | | | |
| MEAN | 181.2 | 171.5 | | | | | | | | | | | | | |
| S.D. | 7.4 | 10.6 | | | | | | | | | | | | | |

(D) - ANIMAL DEAD

LAC912-09/04 LAC912P/2 RUN DATE: 22/01/97

HOECHST MARION ROUSSEL SECTION: PRECL.DEV.GERMANY, DRUG SAFETY

INDIVIDUAL BODY WEIGHT GAINS(g) PAGE: 2

STUDY NO: AR1171 TITLE: STUDY NO. 96.0848 ORAL ADMINISTRATION

GROUP: 2 COMPOUND : A00063/001 DOSE: 315 MG/KG

WEIGHT GAINS ARE BETWEEN DAY 1 AND THE DAYS PRINTED

| ANIMAL NUMBER | DAY 1 | DAY 2 | DAY 3 | DAY 4 | DAY 5 | DAY 6 | DAY 7 | DAY 8 | DAY 9 | DAY 10 | DAY 11 | DAY 12 | DAY 13 | DAY 14 | DAY 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MALES | | | | | | | | | | | | | | | |
| 11 | 0 | - | - | - | - | - | - | 42 | - | - | - | - | - | - | 115 (D) |
| 12 | 0 | - | - | - | - | - | - | 55 | - | - | - | - | - | - | 84 (D) |
| 13 | 0 | - | - | - | - | - | - | 56 | - | - | - | - | - | - | 102 (D) |
| 14 | 0 | - | - | - | - | - | - | 49 | - | - | - | - | - | - | 72 (D) |
| 15 | 0 | - | - | - | - | - | - | 57 | - | - | - | - | - | - | 96 (D) |
| MEAN | | | | | | | | 51.8 | | | | | | | 93.8 |
| S.D. | | | | | | | | 6.3 | | | | | | | 16.5 |

LAC912-09/04 LAC912P/2 RUN DATE: 22/01/97

HOECHST MARION ROUSSEL SECTION: PRECL.DEV.GERMANY, DRUG SAFETY

INDIVIDUAL BODY WEIGHT GAINS(g) PAGE: 1

STUDY NO: AR1171 TITLE: STUDY NO. 96.0848 ORAL ADMINISTRATION

GROUP: 1 COMPOUND : A00063/001 DOSE: 400 MG/KG

WEIGHT GAINS ARE BETWEEN DAY 1 AND THE DAYS PRINTED

| ANIMAL NUMBER | DAY 1 | DAY 2 | DAY 3 | DAY 4 | DAY 5 | DAY 6 | DAY 7 | DAY 8 | DAY 9 | DAY 10 | DAY 11 | DAY 12 | DAY 13 | DAY 14 | DAY 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MALES | | | | | | | | | | | | | | | |
| 1 | 0 | - | - | - | - | - | - | 51 | - | - | - | - | - | - | 100 (D) |
| 2 | 0 (D) | | | | | | | | | | | | | | |
| 3 | 0 | - | - | - | - | - | - | 51 | - | - | - | - | - | - | 125 (D) |
| 4 | 0 (D) | | | | | | | | | | | | | | |
| 5 | 0 (D) | | | | | | | | | | | | | | |
| MEAN | | | | | | | | 51.0 | | | | | | | 112.5 |
| S.D. | | | | | | | | 0.0 | | | | | | | 17.7 |
| FEMALES | | | | | | | | | | | | | | | |
| 6 | 0 (D) | | | | | | | | | | | | | | |
| 7 | 0 (D) | | | | | | | | | | | | | | |
| 8 | 0 | - | - | - | - | - | - | 36 | - | - | - | - | - | - | 66 (D) |
| 9 | 0 | - | - | - | - | - | - | -7 | - | - | - | - | - | - | 23 (D) |
| 10 | 0 | - | - | - | - | - | - | 33 | - | - | - | - | - | - | 56 (D) |
| MEAN | | | | | | | | 20.7 | | | | | | | 48.3 |
| S.D. | | | | | | | | 24.0 | | | | | | | 22.5 |

LAC912-09/04 LAC912P/2 RUN DATE: 22/01/97

HOECHST MARION ROUSSEL SECTION: PRECL.DEV.GERMANY, DRUG SAFETY

INDIVIDUAL BODY WEIGHT GAINS(g) PAGE: 3

STUDY NO: AR1171 TITLE: STUDY NO. 96.0848 ORAL ADMINISTRATION

GROUP: 3 COMPOUND : A00063/001 DOSE: 500 MG/KG

WEIGHT GAINS ARE BETWEEN DAY 1 AND THE DAYS PRINTED

| ANIMAL NUMBER | DAY 1 | DAY 2 | DAY 3 | DAY 4 | DAY 5 | DAY 6 | DAY 7 | DAY 8 | DAY 9 | DAY 10 | DAY 11 | DAY 12 | DAY 13 | DAY 14 | DAY 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MALES | | | | | | | | | | | | | | | |
| 16 | 0 (D) | | | | | | | | | | | | | | |
| 17 | 0 (D) | | | | | | | | | | | | | | |
| 18 | 0 | -12 (D) | | | | | | | | | | | | | |
| 19 | 0 | -9 (D) | | | | | | | | | | | | | |
| 20 | 0 (D) | | | | | | | | | | | | | | |
| MEAN | | -10.5 | | | | | | | | | | | | | |
| S.D. | | 2.1 | | | | | | | | | | | | | |

LAC700-02/01 LAC700P/2 RUN DATE: 22/01/97

HOECHST MARION ROUSSEL SECTION: PRECL.DEV.GERMANY, DRUG SAFETY

CLINICAL OBSERVATIONS TIME TO RESPONSE PRINT PAGE: 2
[ RELATIVE TIME AFTER FIRST DOSE ]

STUDY NUMBER: AR1171 TITLE: STUDY NO. 96.0848 ORAL ADMINISTRATION COMPOUND NUMBER : A00063/001

GROUP: 2
DOSE LEVEL: 315 DOSE UNIT: MG/KG SEX: MALE

| DAY NUMBER<br>START TIME<br>FINISH TIME | 1<br>00 MI<br>10 MI | 1<br>10 MI<br>30 MI | 1<br>30 MI<br>60 MI | 1<br>01 HR<br>02 HR | 1<br>02 HR<br>04 HR | 1<br>04 HR<br>06 HR | 1<br>06 HR<br>08 HR |
|---|---|---|---|---|---|---|---|
| NO. OF ANIMALS DOSED | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| NO. OF ANIMALS SURVIVED | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| NO ABNORMALITIES DETECTED | 5 | - | - | - | - | - | - |
| INCREASED RESPIRAT.RATE | - | 5 | 5 | 5 | 5 | 5 | 5 |
| PANTING | - | - | - | - | - | - | 1 |
| IRREGULAR RESPIRATION | - | 5 | 5 | 5 | 5 | 5 | 4 |
| STUPOR | - | - | - | - | - | 1 | 2 |
| COAT BRISTLING | - | 1 | 1 | 2 | 3 | 4 | 4 |
| FLANKS DRAWN IN | - | - | - | - | 1 | 1 | 1 |
| STILTED GAIT | - | 5 | 5 | 5 | 5 | 4 | 4 |
| UNCOORDINATED GAIT | - | 1 | 4 | 4 | 4 | 4 | 4 |
| PALE SKIN | - | - | - | - | - | 2 | 3 |
| PRONE POSITION | - | - | - | - | - | 1 | 1 |
| SQUATTING POSTURE | - | 5 | 5 | 5 | 5 | 4 | 4 |
| TONOCLONIC CONVULSIONS | - | - | 1 | 2 | 2 | 3 | 2 |
| TREMBLING | - | 1 | 1 | 1 | 1 | 1 | 1 |
| PALPEBR.FISSURE NARROW | - | 2 | 3 | 4 | 4 | 5 | 5 |
| DECR.SPONTANEOUS ACTIVITY | - | 5 | 5 | 5 | 5 | 4 | 3 |

LAC700-02/01 LAC700P/2 RUN DATE: 22/01/97

HOECHST MARION ROUSSEL SECTION: PRECL.DEV.GERMANY, DRUG SAFETY

C L I N I C A L O B S E R V A T I O N S T I M E T O R E S P O N S E P R I N T PAGE: 3
[ ABSOLUTE TIME AFTER ANIMAL START DATE ]

STUDY NUMBER: AR1171 TITLE: STUDY NO. 96.0848 ORAL ADMINISTRATION COMPOUND NUMBER : A00063/001

GROUP: 2
DOSE LEVEL: 315 DOSE UNIT: MG/KG SEX: MALE

| DAY NUMBER | 2 | 2 | 3 | 3 | 4 | 4 | 5 | 5 | 6 | 6 |
|---|---|---|---|---|---|---|---|---|---|---|
| START TIME | 07:00 | 12:00 | 07:00 | 12:00 | 07:00 | 12:00 | 07:00 | 12:00 | 07:00 | 12:00 |
| FINISH TIME | 12:00 | 16:00 | 12:00 | 16:00 | 12:00 | 16:00 | 12:00 | 16:00 | 12:00 | 16:00 |
| NO. OF ANIMALS DOSED | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| NO. OF ANIMALS SURVIVED | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| NO ABNORMALITIES DETECTED | - | - | 1 | 1 | 5 | - | 5 | - | 5 | 5 |
| INCREASED RESPIRAT.RATE | 4 | - | - | - | - | - | - | - | - | - |
| FLANKS DRAWN IN | 1 | - | - | - | - | - | - | - | - | - |
| STILTED GAIT | 2 | - | - | - | - | - | - | - | - | - |
| UNCOORDINATED GAIT | 5 | 5 | 4 | 4 | - | - | - | - | - | - |
| PALE SKIN | 2 | 2 | - | - | - | - | - | - | - | - |
| SQUATTING POSTURE | 5 | 2 | 1 | 1 | - | - | - | - | - | - |
| DIARRHEA | 1 | 1 | - | - | - | - | - | - | - | - |

LAC700-02/01 LAC700P/2 RUN DATE: 22/01/97

HOECHST MARION ROUSSEL SECTION: PRECL.DEV.GERMANY, DRUG SAFETY

C L I N I C A L O B S E R V A T I O N S T I M E T O R E S P O N S E P R I N T PAGE: 4
[ ABSOLUTE TIME AFTER ANIMAL START DATE ]

STUDY NUMBER: AR1171 TITLE: STUDY NO. 96.0848 ORAL ADMINISTRATION COMPOUND NUMBER : A00063/001

GROUP: 2
DOSE LEVEL: 315 DOSE UNIT: MG/KG SEX: MALE

| DAY NUMBER<br>START TIME<br>FINISH TIME | 7<br>07:00<br>16:00 | 8<br>07:00<br>16:00 | 9<br>07:00<br>16:00 | 10<br>07:00<br>16:00 | 11<br>07:00<br>16:00 | 12<br>07:00<br>16:00 | 13<br>07:00<br>16:00 | 14<br>07:00<br>16:00 | 15<br>07:00<br>16:00 |
|---|---|---|---|---|---|---|---|---|---|
| NO. OF ANIMALS DOSED<br>NO. OF ANIMALS SURVIVED | 5<br>5 | 5<br>5 | 5<br>5 | 5<br>5 | 5<br>5 | 5<br>5 | 5<br>5 | 5<br>5 | 5<br>0 |
| NO ABNORMALITIES DETECTED<br>KILLED AT END OF STUDY<br>DISSECTION:NO MACR.FINDG. | 5<br>- | 5<br>- | 5<br>- | 5<br>- | 5<br>- | 5<br>- | 5<br>- | 5<br>- | -<br>5<br>5 |

LAC700-02/01 LAC700P/2 RUN DATE: 22/01/97

HOECHST MARION ROUSSEL SECTION: PRECL.DEV.GERMANY, DRUG SAFETY

C L I N I C A L O B S E R V A T I O N S T I M E T O R E S P O N S E P R I N T PAGE: 1
[ RELATIVE TIME AFTER FIRST DOSE ]

STUDY NUMBER: AR1171 TITLE: STUDY NO. 96.0848 ORAL ADMINISTRATION COMPOUND NUMBER : A00063/001

GROUP: 1
DOSE LEVEL: 400 DOSE UNIT: MG/KG SEX: MALE

| DAY NUMBER<br>START TIME<br>FINISH TIME | 1<br>00 MI<br>10 MI | 1<br>10 MI<br>30 MI | 1<br>30 MI<br>60 MI | 1<br>01 HR<br>02 HR | 1<br>02 HR<br>04 HR | 1<br>04 HR<br>06 HR | 1<br>06 HR<br>08 HR |
|---|---|---|---|---|---|---|---|
| NO. OF ANIMALS DOSED | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| NO. OF ANIMALS SURVIVED | 5 | 5 | 5 | 2 | 2 | 2 | 2 |
| NO ABNORMALITIES DETECTED | 5 | - | - | - | - | - | - |
| FOUND DEAD | - | - | - | 3 | - | - | - |
| STRADDLING HIND LIMBS | - | - | - | - | - | 2 | 2 |
| IRREGULAR RESPIRATION | - | 5 | 5 | 2 | 2 | 1 | - |
| STUPOR | - | - | 4 | 1 | 2 | 2 | 2 |
| COAT BRISTLING | - | - | - | 1 | 2 | 2 | 2 |
| STILTED GAIT | - | - | - | 1 | - | - | - |
| ATAXIC GAIT | - | - | 3 | - | 1 | 2 | 2 |
| UNCOORDINATED GAIT | - | 5 | 3 | 2 | 1 | - | - |
| PALE SKIN | - | - | - | - | - | 2 | 2 |
| SQUATTING POSTURE | - | 5 | 5 | 2 | 2 | 2 | 2 |
| TONOCLONIC CONVULSIONS | - | - | 4 | 1 | 2 | 2 | 2 |
| TREMBLING | - | 3 | 1 | 1 | - | - | - |
| PALPEBR.FISSURE NARROW | - | - | 4 | 1 | 2 | 2 | 2 |
| DECR.SPONTANEOUS ACTIVITY | - | - | 1 | 1 | - | - | - |

LAC700-02/01 LAC700P/2 RUN DATE: 22/01/97

HOECHST MARION ROUSSEL SECTION: PRECL.DEV.GERMANY, DRUG SAFETY

C L I N I C A L O B S E R V A T I O N S T I M E T O R E S P O N S E P R I N T PAGE: 1
[ ABSOLUTE TIME AFTER ANIMAL START DATE ]

STUDY NUMBER: AR1171 TITLE: STUDY NO. 96.0848 ORAL ADMINISTRATION COMPOUND NUMBER : A00063/001

GROUP: 1
DOSE LEVEL: 400 DOSE UNIT: MG/KG SEX: MALE

| DAY NUMBER<br>START TIME<br>FINISH TIME | 2<br>07:00<br>12:00 | 2<br>12:00<br>16:00 | 3<br>07:00<br>12:00 | 3<br>12:00<br>16:00 | 4<br>07:00<br>12:00 | 4<br>12:00<br>16:00 | 5<br>07:00<br>12:00 | 5<br>12:00<br>16:00 | 6<br>07:00<br>12:00 | 6<br>12:00<br>16:00 |
|---|---|---|---|---|---|---|---|---|---|---|
| NO. OF ANIMALS DOSED | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| NO. OF ANIMALS SURVIVED | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| NO ABNORMALITIES DETECTED | - | - | - | - | 1 | - | 2 | 2 | 2 | - |
| STRADDLING HIND LIMBS | 1 | - | - | - | - | - | - | - | - | - |
| COAT BRISTLING | 1 | 1 | - | - | - | - | - | - | - | - |
| STILTED GAIT | 2 | 1 | - | - | - | - | - | - | - | - |
| UNCOORDINATED GAIT | 2 | 2 | 2 | - | 1 | - | - | - | - | - |
| SQUATTING POSTURE | 2 | 2 | 1 | - | - | - | - | - | - | - |

LAC700-02/01 LAC700P/2

RUN DATE: 22/01/97

HOECHST MARION ROUSSEL

SECTION: PRECL.DEV.GERMANY, DRUG SAFETY

C L I N I C A L  O B S E R V A T I O N S  T I M E  T O  R E S P O N S E  P R I N T

PAGE: 2

( ABSOLUTE TIME AFTER ANIMAL START DATE )

STUDY NUMBER: AR1171

TITLE: STUDY NO. 96.0848 ORAL ADMINISTRATION

COMPOUND NUMBER : A00063/001

GROUP: 1
DOSE LEVEL: 400

DOSE UNIT: MG/KG

SEX: MALE

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| DAY NUMBER | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| START TIME | 07:00 | 07:00 | 07:00 | 07:00 | 07:00 | 07:00 | 07:00 | 07:00 | 07:00 |
| FINISH TIME | 16:00 | 16:00 | 16:00 | 16:00 | 16:00 | 16:00 | 16:00 | 16:00 | 16:00 |
| NO. OF ANIMALS DOSED | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| NO. OF ANIMALS SURVIVED | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 0 |
| NO ABNORMALITIES DETECTED | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | - |
| KILLED AT END OF STUDY | - | - | - | - | - | - | - | - | 2 |
| DISSECTION:NO MACR.FINDG. | - | - | - | - | - | - | - | - | 2 |

LAC700-02/01 LAC700P/2 RUN DATE: 22/01/97

HOECHST MARION ROUSSEL SECTION: PRECL.DEV.GERMANY, DRUG SAFETY

C L I N I C A L O B S E R V A T I O N S T I M E T O R E S P O N S E P R I N T PAGE: 3
[ RELATIVE TIME AFTER FIRST DOSE ]

STUDY NUMBER: AR1171 TITLE: STUDY NO. 96.0848 ORAL ADMINISTRATION COMPOUND NUMBER : A00063/001

GROUP: 3
DOSE LEVEL: 500 DOSE UNIT: MG/KG SEX: MALE

| DAY NUMBER<br>START TIME<br>FINISH TIME | 1<br>00 MI<br>10 MI | 1<br>10 MI<br>30 MI | 1<br>30 MI<br>60 MI | 1<br>01 HR<br>02 HR | 1<br>02 HR<br>04 HR | 1<br>04 HR<br>06 HR | 1<br>06 HR<br>08 HR |
|---|---|---|---|---|---|---|---|
| NO. OF ANIMALS DOSED | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| NO. OF ANIMALS SURVIVED | 5 | 5 | 4 | 4 | 3 | 2 | 2 |
| NO ABNORMALITIES DETECTED | 5 | - | - | - | - | - | - |
| FOUND DEAD | - | - | 1 | - | 1 | 1 | - |
| INCREASED RESPIRAT.RATE | - | 5 | 4 | 4 | 3 | 3 | 2 |
| RESPIRATORY SOUNDS | - | 1 | - | - | - | - | - |
| PANTING | - | - | - | - | 1 | 1 | 2 |
| STUPOR | - | 1 | - | 1 | 2 | 3 | 2 |
| COAT BRISTLING | - | - | - | 2 | 3 | 3 | 2 |
| FLANKS DRAWN IN | - | - | 1 | 1 | 1 | 1 | - |
| STILTED GAIT | - | 2 | 3 | 2 | 2 | 1 | - |
| ATAXIC GAIT | - | 1 | - | - | 2 | 2 | 1 |
| UNCOORDINATED GAIT | - | 4 | 4 | 3 | 1 | - | - |
| PALE SKIN | - | 1 | 1 | 2 | 3 | 3 | 2 |
| PRONE POSITION | - | 1 | - | 1 | - | 1 | 1 |
| SQUATTING POSTURE | - | 4 | 4 | 3 | 3 | 2 | 1 |
| SALTATORY & ROLLING CONV. | - | 1 | - | - | - | 1 | 1 |
| TONOCLONIC CONVULSIONS | - | 1 | 1 | 2 | 2 | 2 | 1 |
| TREMBLING | - | 1 | 1 | 2 | 1 | - | - |
| PALPEBR.FISSURE NARROW | - | 1 | 1 | 2 | 2 | 3 | 2 |
| DECR.SPONTANEOUS ACTIVITY | - | 3 | 4 | 3 | 1 | - | - |

LAC700-02/01 LAC700P/2

RUN DATE: 22/01/97

HOECHST MARION ROUSSEL

SECTION: PRECL.DEV.GERMANY, DRUG SAFETY

C L I N I C A L  O B S E R V A T I O N S  T I M E  T O  R E S P O N S E  P R I N T

[ ABSOLUTE TIME AFTER ANIMAL START DATE ]



STUDY NUMBER: AR1171 TITLE: STUDY NO. 96.0848 ORAL ADMINISTRATION COMPOUND NUMBER : A00063/001

GROUP: 3
DOSE LEVEL: 500 DOSE UNIT: MG/KG SEX: MALE

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| DAY NUMBER | 2 | 2 | 3 | 3 | 4 | 4 | 5 | 5 | 6 | 6 |
| START TIME | 07:00 | 12:00 | 07:00 | 12:00 | 07:00 | 12:00 | 07:00 | 12:00 | 07:00 | 12:00 |
| FINISH TIME | 12:00 | 16:00 | 12:00 | 16:00 | 12:00 | 16:00 | 12:00 | 16:00 | 12:00 | 16:00 |
| NO. OF ANIMALS DOSED | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| NO. OF ANIMALS SURVIVED | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| FOUND DEAD | 2 | - | - | - | - | - | - | - | - | - |

LAC700-02/01 LAC700P/2

RUN DATE: 22/01/97

HOECHST MARION ROUSSEL

SECTION: PRECL.DEV.GERMANY, DRUG SAFETY

C L I N I C A L  O B S E R V A T I O N S  T I M E  T O  R E S P O N S E  P R I N T

[ RELATIVE TIME AFTER FIRST DOSE ]

PAGE: 1

STUDY NUMBER: AR1171  TITLE: STUDY NO. 96.0848 ORAL ADMINISTRATION  COMPOUND NUMBER : A00063/001

GROUP: 1
DOSE LEVEL: 400  DOSE UNIT: MG/KG  SEX: FEMALE

| DAY NUMBER<br>START TIME<br>FINISH TIME | 1<br>00 MI<br>10 MI | 1<br>10 MI<br>30 MI | 1<br>30 MI<br>60 MI | 1<br>01 HR<br>02 HR | 1<br>02 HR<br>04 HR | 1<br>04 HR<br>06 HR | 1<br>06 HR<br>08 HR |
|---|---|---|---|---|---|---|---|
| NO. OF ANIMALS DOSED | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| NO. OF ANIMALS SURVIVED | 5 | 5 | 5 | 4 | 3 | 3 | 3 |
| NO ABNORMALITIES DETECTED | 5 | - | - | - | - | - | - |
| FOUND DEAD | - | - | - | 1 | 1 | - | - |
| RESPIRATORY SOUNDS | - | - | - | - | - | - | 1 |
| STRADDLING HIND LIMBS | - | - | - | - | - | - | 2 |
| PANTING | - | - | - | 2 | 1 | - | - |
| IRREGULAR RESPIRATION | - | 5 | 5 | 2 | 2 | 2 | 2 |
| STUPOR | - | - | 5 | 4 | 1 | 1 | 2 |
| COAT BRISTLING | - | - | 1 | 1 | 1 | 1 | 2 |
| FLANKS DRAWN IN | - | - | - | - | 1 | 1 | 1 |
| STILTED GAIT | - | 2 | 2 | 1 | 1 | 1 | 2 |
| ATAXIC GAIT | - | - | 3 | 2 | 1 | 1 | 2 |
| UNCOORDINATED GAIT | - | 5 | 1 | 1 | 2 | 2 | 1 |
| PALE SKIN | - | - | - | - | - | 1 | 2 |
| PRONE POSITION | - | - | 1 | 1 | - | - | - |
| SQUATTING POSTURE | - | 5 | 4 | 3 | 3 | 3 | 3 |
| TONOCLONIC CONVULSIONS | - | - | 5 | 4 | 1 | 1 | 1 |
| TREMBLING | - | 5 | - | - | - | 2 | 2 |
| PALPEBR.FISSURE NARROW | - | - | 5 | 4 | 1 | 1 | 2 |
| DECR.SPONTANEOUS ACTIVITY | - | - | - | - | 2 | 2 | 1 |

LAC700-02/01 LAC700P/2 RUN DATE: 22/01/97

HOECHST MARION ROUSSEL SECTION: PRECL.DEV.GERMANY, DRUG SAFETY

C L I N I C A L O B S E R V A T I O N S T I M E - T O R E S P O N S E P R I N T PAGE: 1
[ ABSOLUTE TIME AFTER ANIMAL START DATE ]

STUDY NUMBER: AR1171 TITLE: STUDY NO. 96.0848 ORAL ADMINISTRATION COMPOUND NUMBER : A00063/001

GROUP: 1
DOSE LEVEL: 400 DOSE UNIT: MG/KG SEX: FEMALE

| DAY NUMBER<br>START TIME<br>FINISH TIME | 2<br>07:00<br>12:00 | 2<br>12:00<br>16:00 | 3<br>07:00<br>12:00 | 3<br>12:00<br>16:00 | 4<br>07:00<br>12:00 | 4<br>12:00<br>16:00 | 5<br>07:00<br>12:00 | 5<br>12:00<br>16:00 | 6<br>07:00<br>12:00 | 6<br>12:00<br>16:00 |
|---|---|---|---|---|---|---|---|---|---|---|
| NO. OF ANIMALS DOSED | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| NO. OF ANIMALS SURVIVED | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| NO ABNORMALITIES DETECTED | - | - | - | - | 2 | - | 2 | 2 | 2 | - |
| RESPIRATORY SOUNDS | 1 | 1 | 1 | - | 1 | - | 1 | 1 | 1 | - |
| STRADDLING HIND LIMBS | 1 | 1 | - | - | - | - | - | - | - | - |
| IRREGULAR RESPIRATION | 2 | 2 | 1 | - | - | - | - | - | - | - |
| COAT BRISTLING | 2 | 1 | - | - | - | - | - | - | - | - |
| FLANKS DRAWN IN | 1 | - | - | - | - | - | - | - | - | - |
| STILTED GAIT | 2 | 1 | 1 | - | - | - | - | - | - | - |
| ATAXIC GAIT | 1 | - | - | - | - | - | - | - | - | - |
| UNCOORDINATED GAIT | 2 | 3 | 3 | - | 1 | - | - | - | - | - |
| SQUATTING POSTURE | 3 | 3 | 1 | - | - | - | - | - | - | - |
| DECR.SPONTANEOUS ACTIVITY | 1 | 2 | - | - | - | - | - | - | - | - |

PAC920-13/00 PAC920P/2 RUN DATE: 22/01/97

HOECHST MARION ROUSSEL SECTION: PRECL.DEV.GERMANY, DRUG SAFETY

P A T H O L O G Y - INTERGROUP COMPARISON OF MACROSCOPIC FINDINGS INCIDENCE PAGE: 1

STUDY NO: AR1171 TITLE: STUDY NO. 96.0848 ORAL ADMINISTRATION

REMOVAL REASON: INTERCURRENT SEX: MALES

| | GROUP 1 400 MG/KG | GROUP 2 315 MG/KG | GROUP 3 500 MG/KG |
|---|---|---|---|
| MALES ON STUDY | 5 | 5 | 5 |
| ANIMALS COMPLETED | 3 | 0 | 5 |
| **LIVER** | | | |
| NO.WITH FINDINGS NO HISTOLOGICAL EXAMINATIONS | 1 | 0 | 4 |
| LIGHT DISCOLORATION | 1 | 0 | 4 |
| SURFACE UNEQUAL | 1 | 0 | 0 |
| **STOMACH** | | | |
| NO.WITH FINDINGS NO HISTOLOGICAL EXAMINATIONS | 0 | 0 | 5 |
| DIFFUSE REDDENING OF MUCOSA | 0 | 0 | 5 |
| DETACHMENT OF THE MUCOSA | 0 | 0 | 1 |
| **SMALL INTESTINE** | | | |
| NO.WITH FINDINGS NO HISTOLOGICAL EXAMINATIONS | 3 | 0 | 5 |
| DIFFUSE REDDENING | 3 | 0 | 5 |
| FULL OF A REDDISH-BLACK MASS, HAEMOCCULT POSITIVE | 3 | 0 | 5 |
| FULL OF YELLOWISH MUCOUS | 0 | 0 | 1 |
| DETACHMENT OF THE MUCOSA | 0 | 0 | 5 |
| **SPLEEN** | | | |
| NO.WITH FINDINGS NO HISTOLOGICAL EXAMINATIONS | 0 | 0 | 4 |
| LIGHT DISCOLORATION | 0 | 0 | 4 |
| **LUNGS** | | | |
| NO.WITH FINDINGS NO HISTOLOGICAL EXAMINATIONS | 3 | 0 | 5 |
| DISCOLORED ORANGE | 3 | 0 | 2 |
| DISCOLORED RED | 0 | 0 | 3 |

PAC920-13/00 PAC920P/2 RUN DATE: 22/01/97

HOECHST MARION ROUSSEL SECTION: PRECL.DEV.GERMANY, DRUG SAFETY

P A T H O L O G Y - INTERGROUP COMPARISON OF MACROSCOPIC FINDINGS INCIDENCE PAGE: 2

STUDY NO: AR1171 TITLE: STUDY NO. 96.0848 ORAL ADMINISTRATION

REMOVAL REASON: INTERCURRENT SEX: FEMALES

| | GROUP 1 400 MG/KG | GROUP 2 315 MG/KG | GROUP 3 500 MG/KG |
|---|---|---|---|
| FEMALES ON STUDY | 5 | 0 | 0 |
| ANIMALS COMPLETED | 2 | 0 | 0 |
| **LIVER** | | | |
| NO.WITH FINDINGS NO HISTOLOGICAL EXAMINATIONS | 1 | 0 | 0 |
| LIGHT DISCOLORATION | 1 | 0 | 0 |
| **SMALL INTESTINE** | | | |
| NO.WITH FINDINGS NO HISTOLOGICAL EXAMINATIONS | 1 | 0 | 0 |
| DIFFUSE REDDENING | 1 | 0 | 0 |
| **LUNGS** | | | |
| NO.WITH FINDINGS NO HISTOLOGICAL EXAMINATIONS | 2 | 0 | 0 |
| DISCOLORED ORANGE | 2 | 0 | 0 |