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September 1990 / bi

Project No.: 10-01-0384-90

**FINAL REPORT**

**Acute Oral Toxicity Test of  
"LEHMANNBLAUSULFAT"**

**in Mice**

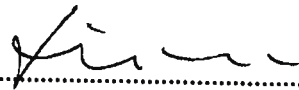
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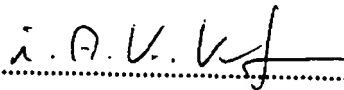
**DECLARATION**

We, the undersigned, hereby declare that the work was performed under our supervision and in accordance with the described procedures. We assure that the reported results faithfully reproduce the raw data obtained during the experimental work. To the best of our knowledge, no circumstances have been left unreported which may have affected the quality or integrity of the data or which might have a potential bearing on the validity and reproducibility of this study.

Dr. med. vet. J. Lindena  
Scientific Supervisor

  
.....

Dr. med. vet. M. Oetjen  
Study Director

  
.....

25.10.90  
.....  
Date



### QUALITY ASSURANCE STATEMENT

The testing facilities utilized in this study have been inspected regularly in accordance with the principles of Good Laboratory Practice for the testing of chemicals as specified by national (BGBl. I, no. 13, § 19 a, March 22, 1990) and international (OECD, Paris, 1982) legislation.

Procedures relevant to this study were inspected periodically. Inspections were conducted according to the standard operating procedures of the testing facility's quality assurance unit. The final report was audited in detail against the approved protocol and all pertinent raw data. The findings of inspections and audits were reported to management and to the study director.

.....*A. Schumann*.....  
(p.p. Quality Assurance Unit)

.....*24.10.1990*.....  
Date

## I. AIM OF THE TEST

The aim of the test was to determine the acute median lethal dosage of "LEHMANNBLAUSULFAT". Information derived from this test serves to indicate the existence of possible hazards likely to arise from short-term exposure to the test article by the oral route.

The test was conducted according to the OECD guideline for the testing of chemicals (OECD 401, February 24, 1987). The principles of Good Laboratory Practice for the testing of chemicals as specified by national (BGBl. I, no. 13, § 19 a, March 22, 1990) and international (OECD, Paris, 1982) legislation were followed during the performance of the study; the IBR standard study protocol, approved and signed on February 28 and March 8, and the corresponding addendum, signed on June 11, were valid.

## II. SUMMARY

The acute oral toxicity of "LEHMANNBLAUSULFAT" was investigated in five dose groups of NMRI mice, each containing 5 males and 5 females.

On the basis of the range finding results, each animal was given a single oral administration of "LEHMANNBLAUSULFAT" at a dose of 125 mg/kg (group I), 250 mg/kg (group II), 500 mg/kg (group III), 750 mg/kg (group IV) and 1000 mg/kg (group V) bodyweight, respectively.

Clinical observations were conducted at regular intervals during the 14-day observation period. Body weights were measured at days 0, 7 and 14. Gross pathological examinations were performed immediately on animals found dead or killed in extremis and at termination on surviving animals. LD<sub>50</sub> values were determined at 24 h and 14 days.

The following results were obtained:

1. Severe abnormal clinical signs were observed with dose related intensity up to 2 days p.a.
2. Pre-terminal deaths occurred in all dose groups with dose related intensity.
3. Reduced weight gains were observed in the surviving animals.
4. Gross pathological examinations at 14 days p.a. (terminal necropsies) revealed no test article-dependent findings, in none of the dose groups. However, all dose animals killed in extremis or died spontaneously revealed alterations which were considered to be test article-related.
5. The following LD<sub>50</sub> values were determined at 24 h:

|                 |   |           |
|-----------------|---|-----------|
| male            | = | 333 mg/kg |
| female          | = | 351 mg/kg |
| male and female | = | 327 mg/kg |
6. According to EEC directive 83/467 EEC and the Gefahrstoffverordnung (GefStoffV), 1987 (BGBl. I, p. 2721), when administered by the oral route, the test article

**"LEHMANNBLAUSULFAT"**

is classified as "slightly toxic".

### III. MATERIAL AND METHOD

#### 1 Animals

**Species:** mouse

**Strain:** BOR: NMRI, white

**Source:** Firma Winkelmann, Versuchstierzucht,  
Gartenstr. 27  
4799 Borchten

**Date of receipt:** June 26, 1990

**Acclimation period:** at least 7 days

**Animal selection:** random

**Animal identification:** colour identification; cage labelled with the  
following information: dosage, sex, date of study  
initiation, project no.

**Weight range at  
study initiation:** m: 24.8 - 37.8 g, f: 21.7 - 29.4 g

#### 2 Husbandry

**Housing:** collective housing up to a maximum of 5 animals  
per cage (Macrolon type III)

**Illumination:** artificial lighting (120 lux) from  
7.00 a.m. - 7.00 p.m.

**Temperature:**  $21 \pm 2^{\circ} \text{C}$

**Relative humidity:** 50 - 85 %

**Measurement:** with thermohygrometer twice daily

**3 Test Article**

**Name:** LEHMANNBLAUSULFAT

**Supplied by:**

**Chemical name:** 1-Methoxy-2-amino-4-(2'-hydroxyethyl)-  
amino-benzol-sulfat

**Physical state:** grey powder

**Batch No.:** not specified by sponsor

**Identification:** labelled, where appropriate, with name test  
article, batch no., name of sponsor, IBR project  
no., date of receipt, storage conditions, handling  
precautions and expiry date

**Storage:** ambient, in the dark

**Stability:** stable

**Solvent/vehicle:** --



#### **4 Test Conditions**

##### **4.1 *Pre-experimental Procedure***

Prior to study initiation, the animals were acclimated to laboratory conditions for at least 7 days.

The animals were fasted from 16 h before until 3 - 4 h after administration of the test article.

##### **4.2 *Administration of the Test Article***

The test article was administered as a 1.25 - 10 % dilution in aqua deion.

A single oral administration of the test article was performed by gavage using a stomach tube.

##### **4.3 *Range Finding***

A preliminary range finding test with a dose of 2000 mg/kg body weight was conducted on two female mice.

#### 4.4 *Main Study*

##### 4.4.1 Clinical Observations

In each animal a number of clinical-toxicological signs were evaluated according to a modified *Irwin-Screening* procedure (*Screening Methods in Pharmacology*, R. A. Turner, 1965, p. 26). Any change from the normal condition was noted (increase or decrease) and the degree of severity of any clinical symptoms was assessed. The animals were examined at the following post-treatment intervals: 10 min, 1 h, 2 h, 6 h, 24 h, and thereafter once daily up to day 14.

##### 4.4.2 Body Weights

The body weights of all animals were recorded immediately before treatment (day 0) and surviving animals were re-weighed on days 7 and 14 (termination).

##### 4.4.3 Necropsy

Animals found dead or killed in extremis were immediately necropsied. The surviving animals were sacrificed by cervical dislocation after 14 days and gross pathological examinations were subsequently performed.

##### 4.4.4 Evaluation of the Data

LD<sub>50</sub> values were calculated according to *Finney D.Y., Probit Analysis*, 3rd edition, Cambridge, 1971.

#### IV. RESULTS

Under the experimental conditions described above, the following results were obtained.

##### Range Finding

There were two deaths in the preliminary study (Appendix 1).

##### Main Study

Doses: Group I: 125 mg/kg; group II: 250 mg/kg; group III: 500 mg/kg; group IV: 750 mg/kg; group V: 1000 mg/kg body weight.

##### Pre-terminal deaths

Cumulative table of pre-terminal deaths:

| Group | dose (mg/kg) | n       | post-treatment time |     |        |     |         |     |
|-------|--------------|---------|---------------------|-----|--------|-----|---------|-----|
|       |              |         | 24 h                |     | 7 days |     | 14 days |     |
|       |              |         | m                   | f   | m      | f   | m       | f   |
| I     | 125          | 5m + 5f | 0/5                 | 0/5 | 1/5    | 1/5 | 1/5     | 1/5 |
| II    | 250          | 5m + 5f | 0/5                 | 0/5 | 2/5    | 1/5 | 2/5     | 1/5 |
| III   | 500          | 5m + 5f | 1/5                 | 0/5 | 4/5    | 3/5 | 4/5     | 3/5 |
| IV    | 750          | 5m + 5f | 1/5                 | 0/5 | 3/5    | 5/5 | 3/5     | 5/5 |
| V     | 1000         | 5m + 5f | 1/5                 | 1/5 | 5/5    | 4/5 | 5/5     | 4/5 |

m = male  
 f = female

Times of individual deaths are listed (Appendix 2).

#### **Clinical signs (Appendix 4)**

Severe clinical symptoms related to CNS-symptoms, coordination, reflexes and autonomic functions were observed with dose related intensity up to 72 h p.a.

#### **Weight gains (Appendix 3)**

Weight gains were reduced in all surviving animals

#### **Necropsy (Appendix 5)**

Gross pathological examinations at 14 days p.a. (terminal necropsies) revealed no test article-dependent findings. Those macroscopic changes observed (Appendix 5) were attributable to the sacrificing procedure or to minor variations which often occur spontaneously in mice of this strain. However, all dose animals killed in extremis or died spontaneously revealed alterations which were considered to be test article-related.

#### **LD<sub>50</sub>**

The following LD<sub>50</sub> values were determined at 14 days:

|                        |          |                  |
|------------------------|----------|------------------|
| <b>male</b>            | <b>=</b> | <b>333 mg/kg</b> |
| <b>female</b>          | <b>=</b> | <b>351 mg/kg</b> |
| <b>male and female</b> | <b>=</b> | <b>327 mg/kg</b> |

**V. GENERAL INFORMATION**

**Study sponsor:**

**Testing facility:**

IBR Forschungs GmbH  
Südkampen Nr. 31

3030 Walsrode 1.

**Scientific supervisor:**

Dr. med. vet. J. Lindena.

**Study director:**

Dr. med. vet. M. Oetjen.

**Responsible technicians:**

I. Becker, M. Händschke.

**Quality assurance:**

Dr. A. Burt, M. Schaardt,  
Dr. A. Stöcker, J. Worthmann,  
Dr. B. Kuszewski, A. Schweneker.

**Experimental period:**

June 6 - August 3, 1990

**Archives:**

All raw data, documentation and specimens, the protocol, and a copy of the final report will be stored in the IBR archives for the minimum period of time specified by current national and international legislation on GLP. Biological specimens will be retained only as long as they afford a meaningful re-evaluation. At the end of the study, any remaining test article will be returned to the sponsor. IBR reserves the right to retain a sample of the test article.















### APPENDIX 3

#### INDIVIDUAL BODY WEIGHTS (g)

Dose: 125 mg/kg

| Anim.<br>No. | Sex | Day 0 | Day 7 | Day 14 |
|--------------|-----|-------|-------|--------|
| 1            | m   | 27.4  | 30.2  | 35.6   |
| 2            | m   | 29.9  | 33.0  | 38.5   |
| 3            | m   | 29.7  | 33.1  | 34.5   |
| 4            | m   | 27.3  | 31.4  | 33.7   |
| 5            | m   | 28.6  | +     | +      |
| 6            | f   | 22.2  | +     | +      |
| 7            | f   | 24.1  | 25.8  | 29.1   |
| 8            | f   | 25.0  | 26.9  | 29.1   |
| 9            | f   | 24.0  | 27.0  | 28.4   |
| 10           | f   | 22.7  | 25.9  | 25.8   |

#### MEAN BODY WEIGHTS (g)

| Sex     | Day 0 | n  | Day 7 | n | Day 14 | n |
|---------|-------|----|-------|---|--------|---|
| males   | 28.6  | 5  | 31.9  | 4 | 35.6   | 4 |
| females | 23.6  | 5  | 26.4  | 4 | 28.1   | 4 |
| m + f   | 26.1  | 10 | 29.2  | 8 | 31.8   | 8 |

### APPENDIX 3

#### INDIVIDUAL BODY WEIGHTS (g)

Dose: 250 mg/kg

| Anim.<br>No. | Sex | Day 0 | Day 7 | Day 14 |
|--------------|-----|-------|-------|--------|
| 1            | m   | 31.3  | 37.6  | 37.8   |
| 2            | m   | 33.1  | 37.8  | 37.4   |
| 3            | m   | 37.8  | 37.5  | 39.9   |
| 4            | m   | 33.4  | +     | +      |
| 5            | m   | 31.7  | +     | +      |
| 6            | f   | 29.4  | 31.3  | 33.8   |
| 7            | f   | 24.5  | 27.0  | 27.2   |
| 8            | f   | 25.5  | +     | +      |
| 9            | f   | 25.9  | 27.1  | 26.7   |
| 10           | f   | 25.1  | 28.3  | 29.0   |

#### MEAN BODY WEIGHTS (g)

| Sex     | Day 0 | n  | Day 7 | n | Day 14 | n |
|---------|-------|----|-------|---|--------|---|
| males   | 33.5  | 5  | 37.6  | 3 | 38.4   | 3 |
| females | 26.1  | 5  | 28.4  | 4 | 29.2   | 4 |
| m + f   | 29.8  | 10 | 32.4  | 7 | 33.1   | 7 |

**APPENDIX 3**

**INDIVIDUAL BODY WEIGHTS (g)**

Dose: 500 mg/kg

| Anim. No. | Sex | Day 0 | Day 7 | Day 14 |
|-----------|-----|-------|-------|--------|
| 1         | m   | 33.6  | +     | +      |
| 2         | m   | 30.9  | 35.7  | 36.2   |
| 3         | m   | 36.3  | +     | +      |
| 4         | m   | 32.7  | +     | +      |
| 5         | m   | 33.0  | +     | +      |
| 6         | f   | 25.5  | +     | +      |
| 7         | f   | 23.3  | 27.0  | 29.7   |
| 8         | f   | 24.0  | +     | +      |
| 9         | f   | 28.4  | +     | +      |
| 10        | f   | 23.4  | 26.7  | 28.3   |

**MEAN BODY WEIGHTS (g)**

| Sex     | Day 0 | n  | Day 7 | n | Day 14 | n |
|---------|-------|----|-------|---|--------|---|
| males   | 33.3  | 5  | 35.7  | 1 | 36.2   | 1 |
| females | 24.9  | 5  | 26.9  | 2 | 29.0   | 2 |
| m + f   | 29.1  | 10 | 29.8  | 3 | 31.4   | 3 |

**APPENDIX 3**

**INDIVIDUAL BODY WEIGHTS (g)**

Dose: 750 mg/kg

| Anim.<br>No. | Sex | Day 0 | Day 7 | Day 14 |
|--------------|-----|-------|-------|--------|
| 1            | m   | 29.6  | 31.7  | 33.3   |
| 2            | m   | 31.0  | +     | +      |
| 3            | m   | 30.3  | +     | +      |
| 4            | m   | 30.9  | +     | +      |
| 5            | m   | 33.5  | 35.5  | 42.6   |
| 6            | f   | 22.3  | +     | +      |
| 7            | f   | 24.1  | +     | +      |
| 8            | f   | 24.0  | +     | +      |
| 9            | f   | 22.0  | +     | +      |
| 10           | f   | 21.7  | +     | +      |

**MEAN BODY WEIGHTS (g)**

| Sex     | Day 0 | n  | Day 7 | n | Day 14 | n |
|---------|-------|----|-------|---|--------|---|
| males   | 31.1  | 5  | 33.6  | 2 | 38.0   | 2 |
| females | 22.8  | 5  | --    | 0 | --     | 0 |
| m + f   | 26.9  | 10 | --    | - | --     | - |

**APPENDIX 3**

**INDIVIDUAL BODY WEIGHTS (g)**

Dose: 750 mg/kg

| Anim.<br>No. | Sex | Day 0 | Day 7 | Day 14 |
|--------------|-----|-------|-------|--------|
| 1            | m   | 24.8  | +     |        |
| 2            | m   | 25.0  | +     |        |
| 3            | m   | 25.4  | +     |        |
| 4            | m   | 26.7  | +     |        |
| 5            | m   | 26.9  | +     |        |
| 6            | f   | 26.8  | 28.9  | 32.4   |
| 7            | f   | 26.8  | +     | +      |
| 8            | f   | 23.5  | +     | +      |
| 9            | f   | 22.1  | +     | +      |
| 10           | f   | 24.5  | +     | +      |

**MEAN BODY WEIGHTS (g)**

| Sex     | Day 0 | n  | Day 7 | n | Day 14 | n |
|---------|-------|----|-------|---|--------|---|
| males   | 25.8  | 5  | --    | - | --     | - |
| females | 24.7  | 5  | 28.9  | 1 | 32.4   | 1 |
| m + f   | 25.3  | 10 | --    | - | --     | - |













## APPENDIX 5

# NECROPSY

Acute Oral Toxicity  
FINAL REPORT, page 30 of 47  
Project no.: 10-01-0384-90

Test article: **LEHMANNBLAUSULFAT**  
Species: **Mouse**  
Sex: **m + f**

Project no.: 10-01-0384-90  
Animal no.: 5m, 6f  
Dose: 125 mg/kg

spontaneous deaths \_\_\_\_\_ hours/days p.a.  
killed in extremis 3 hours/days p.a.  
interim sacrifice \_\_\_\_\_ days p.a.  
terminal sacrifice \_\_\_\_\_ days p.a.

(Note: data is compiled  
only for those animals  
showing specific findings)

Open boxes: no specific findings

| Organs  | Animal no. | Specific Findings                   |
|---|------------|-------------------------------------|
| Brain<br>Spinal cord<br>Peripheral nerve  |            |                                     |
| Lung  |            |                                     |
| Heart<br>Pericardium  |            |                                     |
| Stomach   | 5m,6f      | hyperemic, dilated                  |
| Intestine   | 5m,6f      | hyperemic, dilated                  |
| Liver   |            |                                     |
| Spleen  | 5m,6f      | pale discoloration, reduced in size |
| Kidney<br>left and right  | 5m         | hyperemic pelvis                    |
| Serosa and<br>Vessels   |            |                                     |
| Lymph nodes   |            |                                     |
| Genital system<br>- uterus<br>- vagina cervix<br>- ovary left and right   |            |                                     |
| Genital system<br>- testis left and right<br>- epididymis left and right<br>- seminal vesicle<br>- prostate gland |            |                                     |

APPENDIX 5

NECROPSY **Acute Oral Toxicity**  
**FINAL REPORT, page 31 of 47**  
**Project no.: 10-01-0384-90**

Test article: **LEHMANNBLAUSULFAT**  
 Species: **Mouse**  
 Sex: **m + f**

Project no.: **10-01-0384-90**  
 Animal no.: **m:1-4, f:7-10**  
 Dose: **125 mg/kg**

spontaneous deaths \_\_\_\_\_ hours/days p.a.  
 killed in extremis \_\_\_\_\_ hours/days p.a.  
 interim sacrifice \_\_\_\_\_ days p.a.  
 terminal sacrifice \_\_\_\_\_ 14 days p.a.

(Note: data is compiled  
 only for those animals  
 showing specific findings)

Open boxes: no specific findings

| Organs  | Animal no.    | Specific Findings   |
|---|---------------|---|
| Brain<br>Spinal cord<br>Peripheral nerve  |               |   |
| Lung  |               |   |
| Heart<br>Pericadium   |               |   |
| Stomach   |               |   |
| Intestine   |               |   |
| Liver   |               |   |
| Spleen  |               |   |
| Kidney<br>left and right  | 1m<br>3m, 10f | pale discoloration, thick-skinned surface<br>hyperemic pelvis |
| Serosa and<br>Vessels   |               |   |
| Lymph nodes   |               |   |
| Genital system<br>- uterus<br>- vagina cervix<br>- ovary left and right   |               |   |
| Genital system<br>- testis left and right<br>- epididymis left and right<br>- seminal vesicle<br>- prostate gland |               |   |

APPENDIX 5

NECROPSY

Acute Oral Toxicity  
 FINAL REPORT, page 32 of 47  
 Project no.: 10-01-0384-90

Test article: **LEHMANNBLAUSULFAT**  
 Species: **Mouse**  
 Sex: **m + f**

Project no.: **10-01-0384-90**  
 Animal no.: **m:4+5, f:8**  
 Dose: **250 mg/kg**

spontaneous deaths 48 hours/days p.a.  
 killed in extremis \_\_\_\_\_ hours/days p.a.  
 interim sacrifice \_\_\_\_\_ days p.a.  
 terminal sacrifice \_\_\_\_\_ days p.a.

(Note: data is compiled  
 only for those animals  
 showing specific findings)

Open boxes: no specific findings

| Organs  | Animal no. | Specific Findings |
|---|------------|-------------------|
| Brain<br>Spinal cord<br>Peripheral nerve  |            |                   |
| Lung  |            |                   |
| Heart<br>Pericadium   |            |                   |
| Stomach   |            |                   |
| Intestine   |            |                   |
| Liver   |            |                   |
| Spleen  |            |                   |
| Kidney<br>left and right  |            |                   |
| Serosa and<br>Vessels   |            |                   |
| Lymph nodes   |            |                   |
| Genital system<br>- uterus<br>- vagina cervix<br>- ovary left and right   |            |                   |
| Genital system<br>- testis left and right<br>- epididymis left and right<br>- seminal vesicle<br>- prostate gland |            |                   |



APPENDIX 5

NECROPSY

Acute Oral Toxicity  
 FINAL REPORT, page 33 of 47  
 Project no.: 10-01-0384-90

Test article: LEHMANNBLAUSULFAT  
 Species: Mouse  
 Sex: m + f

Project no.: 10-01-0384-90  
 Animal no.: m:1-3, f:6,7,9,10  
 Dose: 250 mg/kg

spontaneous deaths \_\_\_\_\_ hours/days p.a.  
 killed in extremis \_\_\_\_\_ hours/days p.a.  
 interim sacrifice \_\_\_\_\_ days p.a.  
 terminal sacrifice \_\_\_\_\_ 14 days p.a.

(Note: data is compiled  
 only for those animals  
 showing specific findings)

Open boxes: no specific findings

| Organs  | Animal no. | Specific Findings  |
|---|------------|--------------------|
| Brain<br>Spinal cord<br>Peripheral nerve  |            |                    |
| Lung  |            |                    |
| Heart<br>Pericardium  |            |                    |
| Stomach   |            |                    |
| Intestine   |            |                    |
| Liver   |            |                    |
| Spleen  |            |                    |
| Kidney<br>left and right  | 10f        | dark discoloration |
| Serosa and<br>Vessels   |            |                    |
| Lymph nodes   |            |                    |
| Genital system<br>- uterus<br>- vagina cervix<br>- ovary left and right   |            |                    |
| Genital system<br>- testis left and right<br>- epididymis left and right<br>- seminal vesicle<br>- prostate gland |            |                    |

## APPENDIX 5

# NECROPSY

Acute Oral Toxicity  
FINAL REPORT, page 34 of 47  
Project no.: 10-01-0384-90

Test article: **LEHMANNBLAUSULFAT**  
Species: **Mouse**  
Sex: **m + f**

Project no.: 10-01-0384-90  
Animal no.: m:1,3-5 f:6,8,9  
Dose: 500 mg/kg

spontaneous deaths 24 - 48 hours/days p.a.  
killed in extremis \_\_\_\_\_ hours/days p.a.  
interim sacrifice \_\_\_\_\_ days p.a.  
terminal sacrifice \_\_\_\_\_ days p.a.

(Note: data is compiled  
only for those animals  
showing specific findings)

Open boxes: no specific findings

| Organs  | Animal no.             | Specific Findings                      |
|---|------------------------|--|
| Brain<br>Spinal cord<br>Peripheral nerve  |                        |  |
| Lung  |                        |  |
| Heart<br>Pericardium  |                        |  |
| Stomach   | 1m<br>3m; 6f           | blue discoloration<br>hyperemic        |
| Intestine   | 1m                     | hyperemic                              |
| Liver   | m:1,3-5; f:6,8,9       | dark discoloration                     |
| Spleen  | m:1,3-5; f:6,8,9       | pale discoloration, reduced in size    |
| Kidney<br>left and right  | m:3-5; 6f<br>m:3,4; 9f | hyperemic pelvis<br>pale discoloration |
| Serosa and<br>Vessels   |                        |  |
| Lymph nodes   |                        |  |
| Genital system<br>- uterus<br>- vagina cervix<br>- ovary left and right   | 8f                     | hydrometra                             |
| Genital system<br>- testis left and right<br>- epididymis left and right<br>- seminal vesicle<br>- prostate gland |                        |  |

APPENDIX 5

NECROPSY **Acute Oral Toxicity**  
**FINAL REPORT, page 35 of 47**  
**Project no.: 10-01-0384-90**

Test article: **LEHMANNBLAUSULFAT**  
 Species: **Mouse**  
 Sex: **m + f**

Project no.: **10-01-0384-90**  
 Animal no.: **m:2; f:7,10**  
 Dose: **500 mg/kg**

spontaneous deaths \_\_\_\_\_ hours/days p.a.  
 killed in extremis \_\_\_\_\_ hours/days p.a.  
 interim sacrifice \_\_\_\_\_ days p.a.  
 terminal sacrifice \_\_\_\_\_ **14** days p.a.

(Note: data is compiled  
 only for those animals  
 showing specific findings)

Open boxes: no specific findings

| Organs  | Animal no.       | Specific Findings                              |
|---|------------------|--|
| Brain<br>Spinal cord<br>Peripheral nerve  |                  |  |
| Lung  |                  |  |
| Heart<br>Pericadium   |                  |  |
| Stomach   |                  |  |
| Intestine   |                  |  |
| Liver   |                  |  |
| Spleen  |                  |  |
| Kidney<br>left and right  | <b>2m<br/>7f</b> | <b>hyperemic pelvis<br/>pale discoloration</b> |
| Serosa and<br>Vessels   |                  |  |
| Lymph nodes   |                  |  |
| Genital system<br>- uterus<br>- vagina cervix<br>- ovary left and right   |                  |  |
| Genital system<br>- testis left and right<br>- epididymis left and right<br>- seminal vesicle<br>- prostate gland |                  |  |

APPENDIX 5

NECROPSY **Acute Oral Toxicity**  
**FINAL REPORT, page 36 of 47**  
**Project no.: 10-01-0384-90**

Test article: **LEHMANNBLAUSULFAT**  
 Species: **Mouse**  
 Sex: **m + f**

Project no.: **10-01-0384-90**  
 Animal no.: **m:2-4; f:6-10**  
 Dose: **750 mg/kg**

spontaneous deaths 24 - 72 hours/days p.a.  
 killed in extremis \_\_\_\_\_ hours/days p.a.  
 interim sacrifice \_\_\_\_\_ days p.a.  
 terminal sacrifice \_\_\_\_\_ days p.a.

(Note: data is compiled  
 only for those animals  
 showing specific findings)

Open boxes: no specific findings

| Organs  | Animal no.         | Specific Findings                      |
|---|--------------------|--|
| Brain<br>Spinal cord<br>Peripheral nerve  |                    |  |
| Lung  |                    |  |
| Heart<br>Pericadium   |                    |  |
| Stomach   | f:6-9              | hyperemic, blue discoloration          |
| Intestine   | 2m                 | dilated                                |
| Liver   |                    |  |
| Spleen  | m:2,3; f:6-9       | pale discoloration                     |
| Kidney<br>left and right  | 3m,7f<br>2m, f:8,9 | pale discoloration<br>hyperemic pelvis |
| Serosa and<br>Vessels   |                    |  |
| Lymph nodes   |                    |  |
| Genital system<br>- uterus<br>- vagina cervix<br>- ovary left and right   |                    |  |
| Genital system<br>- testis left and right<br>- epididymis left and right<br>- seminal vesicle<br>- prostate gland |                    |  |

APPENDIX 5

NECROPSY

Acute Oral Toxicity  
 FINAL REPORT, page 37 of 47  
 Project no.: 10-01-0384-90

Test article: LEHMANNBLAUSULFAT  
 Species: Mouse  
 Sex: m + f

Project no.: 10-01-0384-90  
 Animal no.: m: 1, 5  
 Dose: 750 mg/kg

spontaneous deaths \_\_\_\_\_ hours/days p.a.  
 killed in extremis \_\_\_\_\_ hours/days p.a.  
 interim sacrifice \_\_\_\_\_ days p.a.  
 terminal sacrifice \_\_\_\_\_ 14 days p.a.

(Note: data is compiled  
 only for those animals  
 showing specific findings)

Open boxes: no specific findings

| Organs  | Animal no. | Specific Findings  |
|---|------------|--------------------|
| Brain<br>Spinal cord<br>Peripheral nerve  |            |                    |
| Lung  |            |                    |
| Heart<br>Pericardium  |            |                    |
| Stomach   |            |                    |
| Intestine   |            |                    |
| Liver   | m: 1,5     | pale discoloration |
| Spleen  | m: 1,5     | pale discoloration |
| Kidney<br>left and right  | m: 1,5     | pale discoloration |
| Serosa and<br>Vessels   |            |                    |
| Lymph nodes   |            |                    |
| Genital system<br>- uterus<br>- vagina cervix<br>- ovary left and right   |            |                    |
| Genital system<br>- testis left and right<br>- epididymis left and right<br>- seminal vesicle<br>- prostate gland |            |                    |

**APPENDIX 5**

**NECROPSY** Acute Oral Toxicity  
**FINAL REPORT**, page 38 of 47  
 Project no.: 10-01-0384-90

Test article: **LEHMANNBLAUSULFAT**  
 Species: **Mouse**  
 Sex: **m + f**

Project no.: 10-01-0384-90  
 Animal no.: m: 1-5; f: 7-10  
 Dose: 1000 mg/kg

spontaneous deaths 24-48 hours/days p.a. No.: 1-3,5,7,8,10  
 killed in extremis 48 hours/days p.a. No.: 4,9  
 interim sacrifice \_\_\_\_\_ days p.a.  
 terminal sacrifice \_\_\_\_\_ days p.a.

(Note: data is compiled only for those animals showing specific findings)

Open boxes: no specific findings

| Organs  | Animal no.                     | Specific Findings   |
|---|--------------------------------|---|
| Brain<br>Spinal cord<br>Peripheral nerve  |                                |   |
| Lung  |                                |   |
| Heart<br>Pericadium   |                                |   |
| Stomach   | 3m,8f<br>m:1,2,4,5;f:7,9,10    | blue discoloration<br>hyperemic                           |
| Intestine   | m:1,2,5;10f<br>3m,8f           | hyperemic<br>blue discoloration                           |
| Liver   | m:1,2,4,5;9f                   | dark discoloration  |
| Spleen  | m:1,2,4,5;f:9,10               | pale discoloration  |
| Kidney<br>left and right  | m:3,4;f:8,9<br>1m,7f<br>2m,10f | blue discoloration<br>dark discoloration<br>redden pelvis |
| Serosa and<br>Vessels   |                                |   |
| Lymph nodes   |                                |   |
| Genital system<br>- uterus<br>- vagina cervix<br>- ovary left and right   |                                |   |
| Genital system<br>- testis left and right<br>- epididymis left and right<br>- seminal vesicle<br>- prostate gland |                                |   |

**APPENDIX 5**

**NECROPSY** Acute Oral Toxicity  
**FINAL REPORT, page 39 of 47**  
 Project no.: 10-01-0384-90

Test article: **LEHMANNBLAUSULFAT**  
 Species: **Mouse**  
 Sex: **m + f**

Project no.: 10-01-0384-90  
 Animal no.: f: 6  
 Dose: 1000 mg/kg

spontaneous deaths \_\_\_\_\_ hours/days p.a.  
 killed in extremis \_\_\_\_\_ hours/days p.a.  
 interim sacrifice \_\_\_\_\_ days p.a.  
 terminal sacrifice \_\_\_\_\_ 14 days p.a.

(Note: data is compiled  
 only for those animals  
 showing specific findings)

Open boxes: no specific findings

| Organs  | Animal no. | Specific Findings  |
|---|------------|--------------------|
| Brain<br>Spinal cord<br>Peripheral nerve  |            |                    |
| Lung  |            |                    |
| Heart<br>Pericardium  |            |                    |
| Stomach   |            |                    |
| Intestine   |            |                    |
| Liver   |            |                    |
| Spleen  |            |                    |
| Kidney<br>left and right  | 6f         | pale discoloration |
| Serosa and<br>Vessels   |            |                    |
| Lymph nodes   |            |                    |
| Genital system<br>- uterus<br>- vagina cervix<br>- ovary left and right   |            |                    |
| Genital system<br>- testis left and right<br>- epididymis left and right<br>- seminal vesicle<br>- prostate gland |            |                    |

|       |              | 14d M Lehmannblausulfat (Rohstoff) |                  |         |  | 384/ 0-90 |
|-------|--------------|------------------------------------|------------------|---------|--|-----------|
| GROUP | DOSE (MG/KG) | NO. OF SUBJECTS                    | NO. OF RESPONSES | PERCENT |  |           |
| 1     | 125.0000     | 5.                                 | 1.               | 20.0    |  |           |
| 2     | 250.0000     | 5.                                 | 2.               | 40.0    |  |           |
| 3     | 500.0000     | 5.                                 | 4.               | 80.0    |  |           |
| 4     | 750.0000     | 5.                                 | 3.               | 60.0    |  |           |

NO CONFIDENCE LIMIT

| PERCENT | LD (MG/KG) |
|---------|------------|
| 1       | 15.1403    |
| 5       | 37.4504    |
| 10      | 60.6965    |
| 16      | 88.8967    |
| 50      | 333.3795   |
| 84      | 1250.2370  |

A = .629, B = 1.732, CHI = 1.14, G = 1.332

HOMOGENEOUS



| 14d F Lehmannblausulfat (Rohstoff) |              |                 |                  |         |  |  | 384/ 0-90 |
|------------------------------------|--------------|-----------------|------------------|---------|--|--|-----------|
| GROUP                              | DOSE (MG/KG) | NO. OF SUBJECTS | NO. OF RESPONSES | PERCENT |  |  |           |
| 1                                  | 125.0000     | 5.              | 1.               | 20.0    |  |  |           |
| 2                                  | 250.0000     | 5.              | 1.               | 20.0    |  |  |           |
| 3                                  | 500.0000     | 5.              | 3.               | 60.0    |  |  |           |
| 4                                  | 750.0000     | 5.              | 5.               | 100.0   |  |  |           |
| 5                                  | 1000.0000    | 5.              | 4.               | 80.0    |  |  |           |

| PERCENT | LD (MG/KG) | CONFIDENCE LIMIT |            |
|---------|------------|------------------|------------|
|         |            | FROM             | TO         |
| 1       | 44.5517    | .1641            | 121.3392   |
| 5       | 81.5395    | 1.3140           | 177.5187   |
| 10      | 112.5449   | 3.9452           | 219.5745   |
| 16      | 145.1873   | 9.3168           | 262.2271   |
| 50      | 350.7929   | 141.3383         | 627.3228   |
| 84      | 847.5651   | 504.8714         | 6373.4690  |
| 90      | 1093.3910  | 615.6208         | 14741.4400 |

A = -1.606, B = 2.596, CHI = 2.38, G = .509

HOMOGENEOUS

APPENDIX 8

Acute Oral Toxicity  
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| 14d M/F Lehmannblausulfat (Rohstof) |              |                 |                  |         |  |  | 384/ 0-90 |
|-------------------------------------|--------------|-----------------|------------------|---------|--|--|-----------|
| GROUP                               | DOSE (MG/KG) | NO. OF SUBJECTS | NO. OF RESPONSES | PERCENT |  |  |           |
| 1                                   | 125.0000     | 10.             | 2.               | 20.0    |  |  |           |
| 2                                   | 250.0000     | 10.             | 3.               | 30.0    |  |  |           |
| 3                                   | 500.0000     | 10.             | 7.               | 70.0    |  |  |           |
| 4                                   | 750.0000     | 10.             | 8.               | 80.0    |  |  |           |
| 5                                   | 1000.0000    | 10.             | 9.               | 90.0    |  |  |           |

| PERCENT | LD (MG/KG) | CONFIDENCE LIMIT |           |
|---------|------------|------------------|-----------|
|         |            | FROM             | TO        |
| 1       | 36.3327    | 2.4269           | 88.0924   |
| 5       | 69.1443    | 9.1653           | 136.9255  |
| 10      | 97.4438    | 18.5010          | 174.2816  |
| 16      | 127.7912   | 32.0473          | 212.0867  |
| 50      | 326.8658   | 188.5021         | 477.3321  |
| 84      | 836.0618   | 558.3972         | 2133.1730 |
| 90      | 1096.4400  | 692.2582         | 3627.1030 |

A = -1.131, B = 2.438, CHI = .56 , G = .273

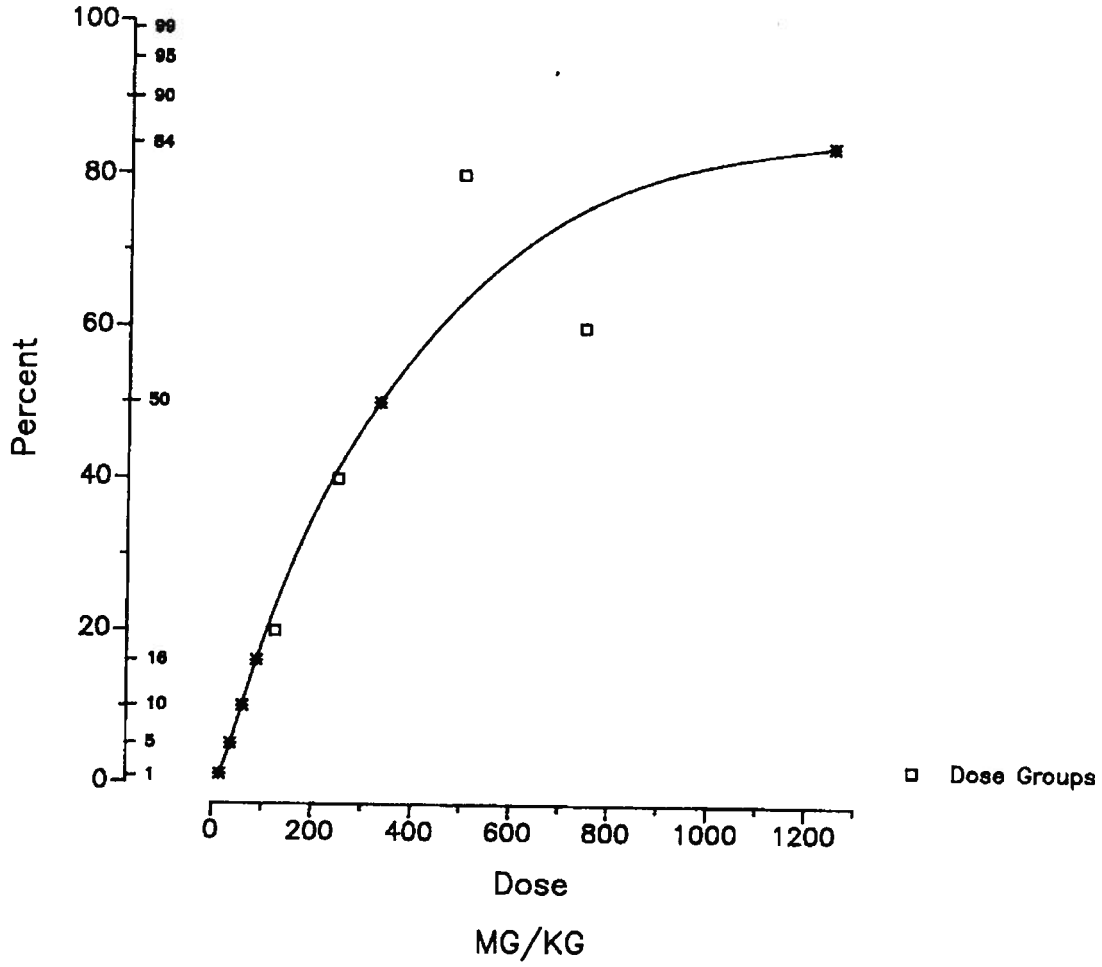
HOMOGENEOUS

APPENDIX 9

Acute Oral Toxicity  
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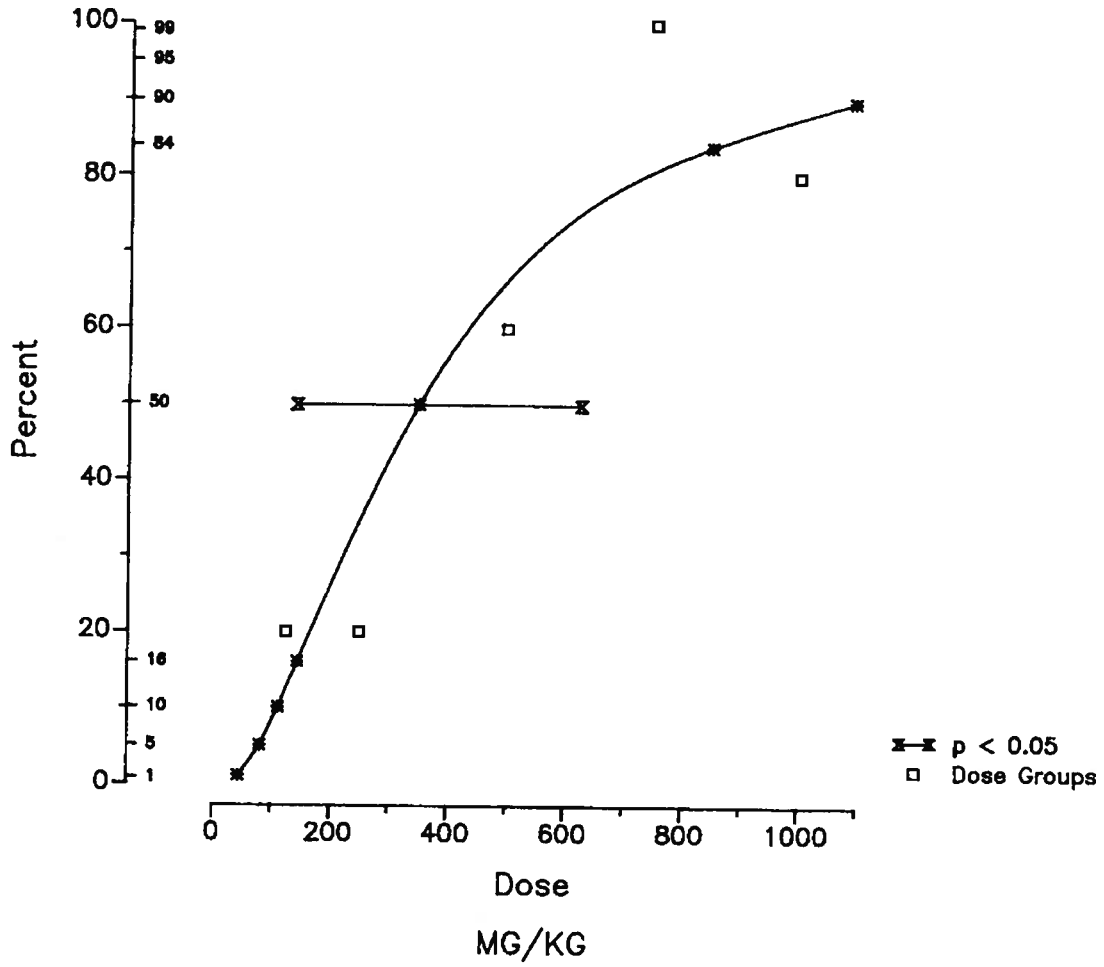
14d M Lehmannblausulfat (Rohstoff)

Proj. 384/ 0-90



14d F Lehmannblausulfat (Rohstoff)

Proj. 384/ 0-90



APPENDIX 12

DIET AND DIET COMPOSITION

Administration: ad libitum

Manufacturer: Ssniff Spezialdiäten GmbH  
4770 Soest/Westfalen

Name: Ssniff-R Alleindiät

Form: pellets, 2.5 cm long, 1.0 cm diameter

Composition (per kg diet):

|               |       |
|---------------|-------|
| Crude protein | 210 g |
| Crude fat     | 30 g  |
| Crude fiber   | 48 g  |
| Crude ash     | 67 g  |

Amino acids (per kg diet):

|               |       |
|---------------|-------|
| Lysine        | 12 g  |
| Methionine    | 3.5 g |
| Cystine       | 3 g   |
| Glycine       | 10 g  |
| Leucine       | 17 g  |
| Isoleucine    | 10 g  |
| Arginine      | 14 g  |
| Phenylalanine | 10 g  |
| Tryptophan    | 3.5 g |
| Histidine     | 5 g   |
| Alanine       | 10 g  |
| Tyrosine      | 7 g   |
| Aspartic acid | 16 g  |
| Glutamic acid | 30 g  |
| Valine        | 10 g  |

Vitamins (supplemented per kg diet):

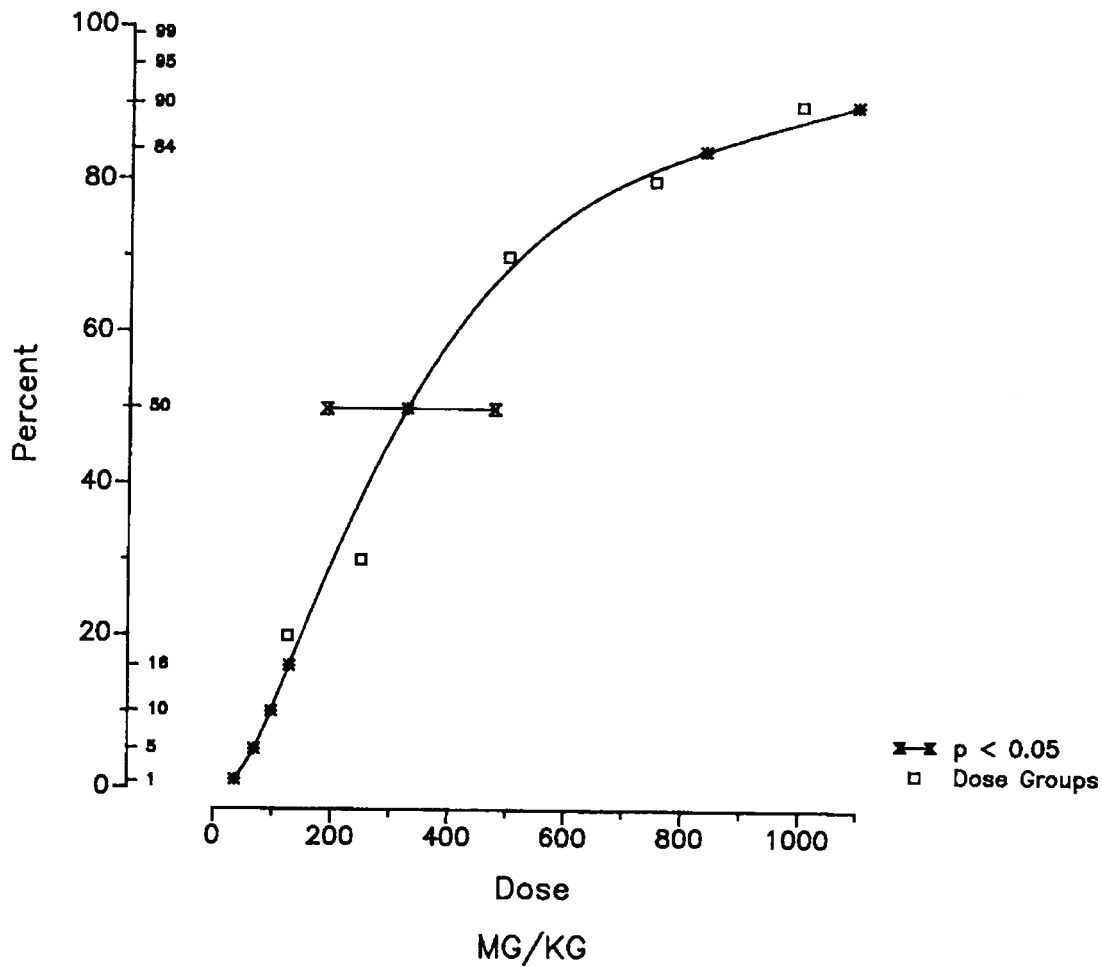
|                  |          |
|------------------|----------|
| A                | 20000 IU |
| D <sub>3</sub>   | 2000 IU  |
| E                | 60 mg    |
| C                | 500 mg   |
| B <sub>1</sub>   | 15 mg    |
| B <sub>2</sub>   | 30 mg    |
| B <sub>6</sub>   | 12 mg    |
| B <sub>12</sub>  | 60 /ug   |
| Biotin           | 200 /ug  |
| Pantothenic acid | 40 mg    |
| Choline          | 1600 mg  |
| Folic acid       | 2 mg     |
| Nicotinic acid   | 120 mg   |
| K <sub>3</sub>   | 5 mg     |

Minerals and trace elements  
(per kg diet):

|            |        |
|------------|--------|
| Calcium    | 11 g   |
| Phosphorus | 8 g    |
| Sodium     | 3 g    |
| Magnesium  | 2 g    |
| Potassium  | 10 g   |
| Manganese  | 75 mg  |
| Copper     | 12 mg  |
| Zinc       | 80 mg  |
| Iodine     | 1 mg   |
| Iron       | 320 mg |
| Fluorine   | 10 mg  |

14d M/F Lehmannblausulfat (Rohstoff)

Proj. 384/ 0-90



**APPENDIX 13**

**WATER**

**Administration:** ad libitum

**System:** Macrolon drinking bottles, Becker & Co.,  
4620 Castrop-Rauxel

**Quality:** drinking water as for human consumption

**Quality control:** half-yearly analytical and bacteriological  
controls (the results of analyses are on file  
at IBR)

GLP-Zertifikat

Bescheinigung

Hiermit wird bestätigt, daß  
die IBR Forschungs GmbH  
in 3030 Walsrode 2,  
Südkampen Nr. 31

am 15.04.1988

von der für die Überwachung  
zuständigen Behörde nach  
Maßgabe der OECD-Richtlinien  
für nationale Inspektionen  
und Überprüfungen über die  
Einhaltung der Grundsätze  
der Guten Laborpraxis  
inspiziert worden ist.

Es wird hiermit bestätigt, daß  
Prüfungen in dieser Prüfein-  
richtung nach den OECD-Grund-  
sätzen für Gute Laborpraxis  
durchgeführt werden.

Lüneburg, 22. 4. 1988

Im Auftrage

*Seippel*

Seippel  
Pharmaziedirektor



Certificate

It is hereby certified that  
the IBR Forschungs GmbH  
in 3030 Walsrode 2,  
Südkampen Nr. 31

on 15.04.1988

was inspected by the competent  
authority in accordance with  
the OECD-Guidelines for  
National GLP Inspections  
and Study Audits regarding  
the compliance with the  
Principles of Good Laboratory  
Practice.

It is hereby certified that  
studies in this test facility  
are conducted in compliance  
with the OECD-Principles of  
Good Laboratory Practice.