Final Report

Residue Analysis of Folpet in Leaf Onions Treated with Folpan 500 SC

Study Director

Dr. Peter Mende

Date

March 17, 1997

Testing Facility

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Study Identification Code

Test substance: Folpan 500 SC
Study code: 96222/01-RFON

Statement of Confidentiality

This report contains confidential and proprietary information of Makhteshim Chemical Works Ltd. which must not be disclosed to anyone except the employees of this company or to persons authorized by law or judicial judgement without the expressed and written approval of Makhteshim Chemical Works Ltd.

Statement of Compliance with the Principles of Good Laboratory Practice

The study described in this report was conducted in compliance with the most recent edition of:

- The Principles of Good Laboratory Practice (GLP), (Chemikaliengesetz, attachment 1, Federal Republic of Germany)
- The OECD Principles of Good Laboratory Practice.

Head of testing facility
(Dr. Hans Eberhardt)

17/03/97 i.V.
Date / Signature

Study director
(Dr. Peter Mende)

17.3.97 P. Mende
Date / Signature

Statement of Quality Assurance Unit

Study code: 96222/01-RFON

Study title: Residue Analysis of Folpet in Leaf Onions Treated with Folpan 500 SC

The conduct of this study or studies of the same type was inspected periodically. Protocol, draft report and final report were audited by the Quality Assurance Unit. The dates are given below:

	Date of audit	Date of report
Study protocol:	02/12/96 11/12/96	02/12/96 –
Experimental phase:	16/12/96	09/01/97
Draft report:	20/01/97	20/01/97
Final report:	27/03/97	27/03/97

Quality assurance manager:
(Jörg Schempf, Dipl.-Ing. (FH))

27 Mar 97
Date / Signature

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1 Summary

1.1 Residue Trial

Residue samples from leaf onions treated with Folpan 500 SC were received from Staatl. Lehr- und Forschungsanstalt für Landwirtschaft, Weinbau und Gartenbau, Neustadt, where the field part of this residue study was performed. The samples consisted of whole plants, i.e. leaves and bulbs. Folpet residues are summarized in Tab. 1.

Tab. 1: Results from residue analysis

Sample ID	Treatment	DAT3	Folpet (mg/kg)
U-RU-MA-03 96NW 0	untreated	0	n.d.
U-RU-MA-03 96NW 14(E)	untreated	14	n.d.
RU-MA-03 96 NW 0	treated	0	5.2
RU-MA-03 96 NW 7	treated	7	4.8
RU-MA-03 96 NW 14(E)	treated	14	1.4
RU-MA-03 96 NW 21	treated	21	1.2

DAT3: days after third treatment
n.d.: not detectable (< 0.01 mg/kg)

1.2 Folpet Analysis

The analysis of folpet was performed in analogy to DFG multimethod S 19 with some modifications:

Extraction:	Cold extraction with acetone
Clean-up:	Partition into dichloromethane, gel-permeation chromatography
Method of determination:	Capillary gas chromatography with electron-capture detection (GC-ECD)
Specifity:	Parent compound
Limit of detection:	0.01 mg/kg
Limit of determination:	0.03 mg/kg
Fortification levels:	0.02 – 5 mg/kg
Recovery ± RSD:	98 ± 16 %

Blanks: In untreated control samples folpet was not detected.

2 Dates

Study initiation date:	09/12/96
Sponsor's agreement to the protocol:	16/12/96
Start of the experimental phase:	12/12/96
End of the experimental phase:	18/12/96
Draft report:	18/12/96
Final report:	17/03/97

3 Study Objective

Obtain data about residues of folpet in leaf onions treated with the fungicidal formulation Folpan 500 SC (active ingredient folpet).

4 Materials and Methods

4.1 Reference Substance

Common name:	Folpet
GAB code:	96222
CAS-No.:	133-07-3
Supplier:	Labor Dr. Ehrenstorfer, Augsburg/Germany
Order No.:	C 138900
Lot No.:	60607
Purity:	99 %
Expiry date:	06/1998
Certificate of analysis:	20/06/96

The reference substance and standard solutions prepared in toluene were stored refrigerated.

4.2 Sample Origin, Storage and Preparation

Field samples were delivered deep-frozen on 02/12/96 from Staatl. Lehr- und Forschungsanstalt für Landwirtschaft, Weinbau und Gartenbau Neustadt, (Dannstadter Str. 91, D-67105 Schifferstadt) to the analytical laboratory of the testing facility (IFU Umweltanalytik GmbH, Bleichstr. 19, D-75173 Pforzheim) where they arrived on 03/12/96. Details on treatment, test substance, trial site etc. are given in the report of the field part of this study (LAUN, 1996).

All samples were stored deep-frozen (< -18 °C) until analysis. Samples were crushed in the frozen state (to avoid decomposition of folpet) and mixed thoroughly. Always the entire sample was processed. An aliquot of this frozen homogenate was taken for immediate analysis (see 4.3.3). The remaining homogenized sample was stored deep-frozen in sealed containers.

4.3 Procedure for Determination of Folpet

Folpet residues were determined by a modified DFG S19 method (SPECHT and TILLKES, 1987).

4.3.1 Apparatus

Round-bottomed flasks, 250 mL
Volumetric flasks, 5 mL
Graduated cylinders, 50 mL, 100 mL and 250 mL
Chromatographic columns, approx. 3 cm i.d.
Laboratory mixer
Rotary vacuum evaporator with water bath (approx. 40 °C)
Gas chromatograph with electron-capture detector
Gel-permeation chromatograph (Abimed Clean-up XL) equipped with chromatographic tube (2.5 cm i.d.)

Round-bottomed flasks and volumetric flasks were rinsed with diluted o-phosphoric acid, water and acetone before use.

4.3.2 Reagents

Acetone
Cyclohexane/ethylacetate (1:1, v/v)
Dichloromethane
Toluene
Extrelut (Merck No. 1.13076)
Glass wool
o-Phosphoric acid, 85 %
Sodium sulfate p.a., anhydrous

4.3.3 Extraction

About 50 g frozen sample (weight W) were accurately weighed and homogenized for several minutes with 150 mL acetone and 0.3 mL o-phosphoric acid in a laboratory mixer. The homogenate was filtered with suction on a Buchner funnel with a paper filter and the filter cake washed slowly with up to 100 mL acetone. The filtrate volume was adjusted to 250 – 300 mL with acetone. Exactly one fifth was taken as aliquot and the acetone evaporated on a rotary vacuum evaporator.

4.3.4 Liquid-liquid Partition

For each sample, a chromatographic column (3 cm i.d.) plugged with glass wool was prepared by filling with 1 cm anhydrous sodium sulfate and 10 g Extrelut. The aqueous residue derived from 4.3.3 was applied on the column, the flask rinsed twice with 10 mL dichloromethane and the rinse applied to the column after 5 mins. Folpet was eluted with 100 mL dichloromethane and the dichloromethane evaporated to near dryness on a vacuum rotary evaporator. Last traces of solvent were removed with a gentle stream of nitrogen.

4.3.5 Gel Permeation Cleanup

The residue obtained from 4.3.4 was dissolved in 5 mL (V_{ex}) cyclohexane/ethylacetate (1:1, v/v) and an aliquot (V_{GPC}) injected into the gel permeation chromatography column (50 g BioRad Bio-Beads S-X3, mobile phase cyclohexane/ethylacetate 1:1, v/v). At a flow rate of 5 mL/min, the fraction from 23 min to 29 min (115 ml to 145 ml elution volume) was collected and the solvent rotary-evaporated to near dryness. Last solvent traces were removed with a gentle stream of nitrogen. The final volume was adjusted to 5 mL (V_{end}) with toluene.

4.3.6 Gas Chromatographic Analysis of Folpet

Gas chromatograph:	Perkin-Elmer Autosystem with built-in autosampler
Column:	Macherey & Nagel OV-101, 25 m x 0.25 mm i.d., 0.25 µm film thickness
Carrier gas:	helium, 70 kPa
Injection:	1 µL splitless at 270 °C
Temperature program:	80 °C, 10 °C/min to 280°C, hold 5 min
Detector:	ECD, 300 °C (nitrogen as make-up gas)
Retention time:	approx. 17.3 min
Quantitation	peak areas, external standards

Samples were injected at least twice. Injections of samples were interspersed with injections of standards to provide a continuous check on the instrument calibration.

4.3.7 Calculation of Folpet Residues

Folpet residues were calculated by the following equation:

$$R = \frac{c \cdot V_{end}}{f_{ex} \cdot W} \cdot \frac{V_{ex}}{V_{GPC}}$$

R residue (mg/kg)

c test substance concentration in final extract, as calculated from the peak areas of the sample vs. standards (µg/mL)

V_{end} final volume of extract (5 mL)

f_{ex} aliquot factor at extraction step (1/5)

W sample weight for analysis (g)

V_{ex} final volume of concentrated crude extract before GPC cleanup (5 mL)

V_{GPC} aliquot of crude extract injected into GPC (mL)

5 Deviations from the Study Protocol

The study title was changed to "Residue Analysis of Folpet in Leaf Onions".

Frozen field samples were crushed manually and then mixed thoroughly instead of homogenizing with a laboratory blendor because the frozen sample material was fragile enough to homogenize by this method.

The rest of the study was performed according to the study protocol dated 29/11/96. This report reflects the conduct of this study.

6 Results

6.1 Method Validation

6.1.1 Recovery

The recovery of folpet was tested by fortification of untreated control samples (mixture of equal weights of both untreated samples) with folpet analytical standard. The mean recovery (± RSD) was 98 ± 16 %. The results are listed in Tab. 2.

Tab. 2: Recovery of folpet from leaf onions

Added (mg/kg)	Found (mg/kg)	Recovery (%)
0	0.005 / 0.007	–
0.02	0.023 / 0.022	115 / 110
0.03	0.036 / 0.029	120 / 97
0.5	0.450	90
1.0	0.892	89
3.5	2.60	74
5.0	4.47	89

6.1.2 Detection Limit and Quantitation Limit

The detection limit was estimated from the signal-to-noise ratio of a recovery sample with the lowest fortification level (0.02 mg/kg). A signal-to-noise ratio of 5:1 correlates to a detection limit of 0.01 mg/kg.

The quantitation limit is defined as the lowest fortification level with a recovery between 70 % and 110 %, with a relative standard deviation not exceeding 20 % and blanks not exceeding 30 %. As indicated by the results given in Tab. 2, the quantitation limit for folpet is 0.03 mg/kg (mean recovery ± RSD: 109 ± 15 %).

6.1.3 Detector Response

The detector response for gas chromatographic analysis of folpet was linear within the range from 0.05 µg/mL to 0.5 µg/mL with $y = 5.9 \cdot 10^5 x - 3400$ and $r^2 = 0.9969$ (Fig. 1).

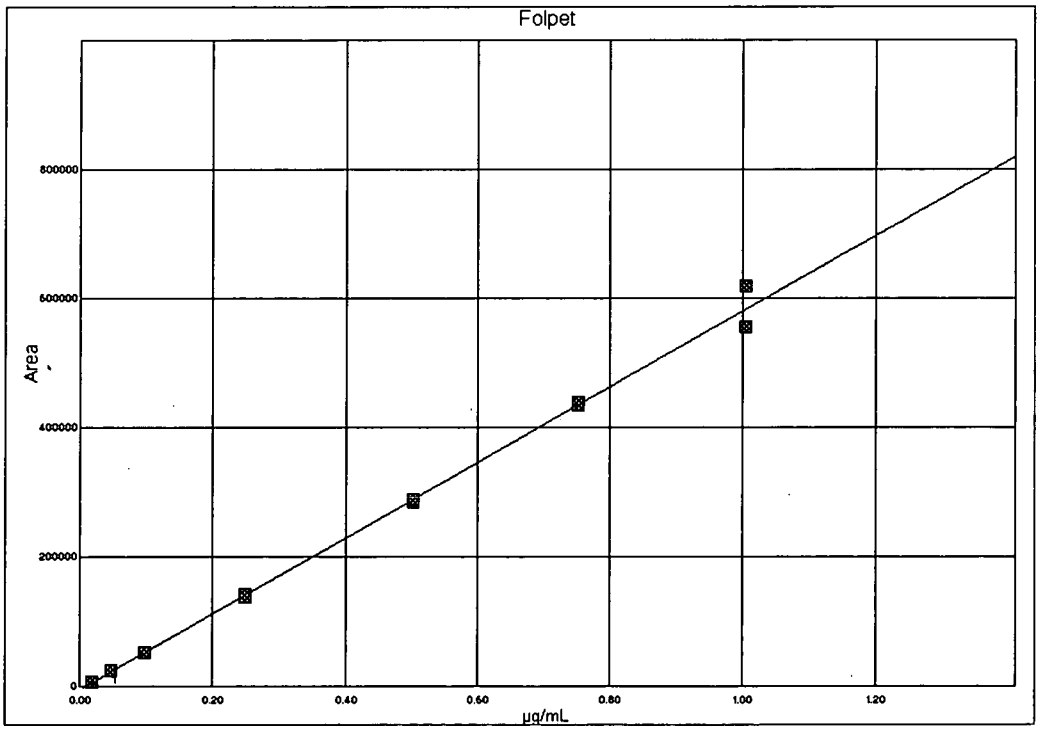

Fig. 1: Linear range of detector response for folpet analysis

6.2 Results from Residue Analysis

The results from residue analysis of leaf onions are given in Tab. 3.

Tab. 3: Results from folpet analysis of onion samples. Results are not corrected for recovery.

Sample ID	Treatment	DAT3	Sampling	Analysis	Folpet (mg/kg)
U-RU-MA-03 96NW 0	untreated	0	18/10/96	12/12/96	n.d.
U-RU-MA-03 96NW 14(E)	untreated	14	01/11/96	12/12/96	n.d.
RU-MA-03 96 NW 0	treated	0	18/10/96	12/12/96	5.2
RU-MA-03 96 NW 7	treated	7	25/10/96	12/12/96	4.8
RU-MA-03 96 NW 14(E)	treated	14	01/11/96	12/12/96	1.4
RU-MA-03 96 NW 21	treated	21	08/11/96	12/12/96	1.2

DAT3: days after third treatment
n.d.: not detectable (< 0.01 mg/kg)

7 Archiving

This study was assigned the study code **96222/01-RFON.** The final report was prepared in two original signed copies. For the periods demanded by the principles of GLP the following documents and materials will be archived:

- Study protocol, raw data, comments of the sponsor on the draft report and one original signed copy of the final report.
- All documentation generated by the Quality Assurance Unit
- A sample of the reference substance.

All documents and materials will be stored in the archives of Arbeitsgemeinschaft GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH. The premises for storing the documents and materials are settled according to the principles of Good Laboratory Practice in the organization of the testing facility.

8 References

LAUN, N., 1996: Rückstandsuntersuchungen zu Folpan 500 SC in Bundzwiebeln 1996 – Feldapplikation, Staatl. Lehr- und Forschungsanstalt für Landwirtschaft, Weinbau und Gartenbau, Neustadt

SPECHT, W., TILLKES, M., 1987: Organochlorine, organophosphorus, nitrogen-containing and other pesticides. Method S 19. In: Manual of Pesticide Residue Analysis, Vol. I, VCH Verlagsgesellschaft, Weinheim/Germany, pp. 383-400.

9 Appendix

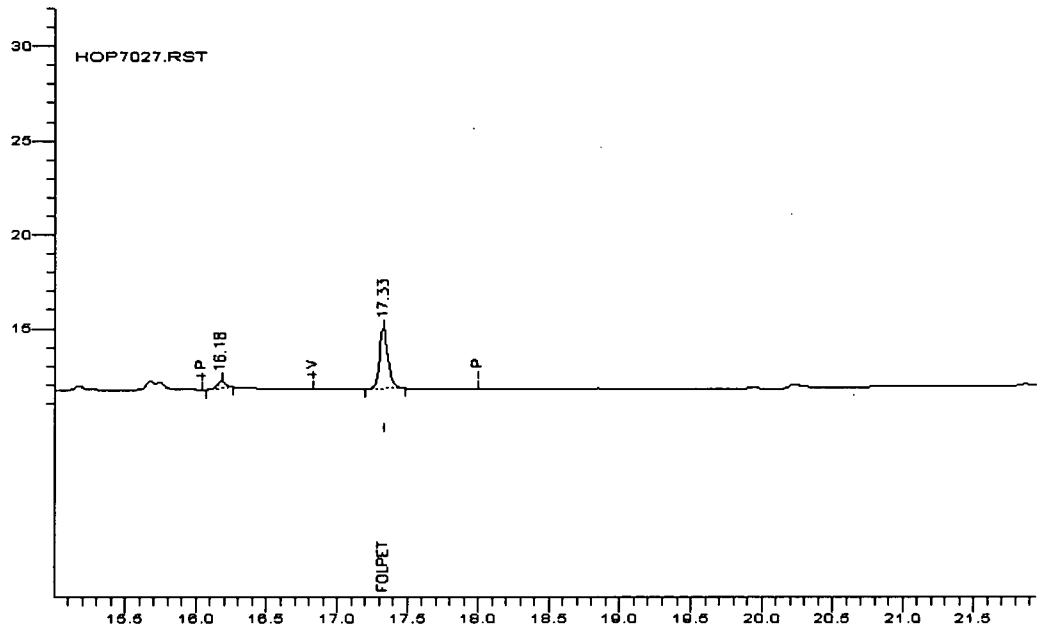

Fig. 2: Chromatogram of a folpet standard (0.05 µg/mL)

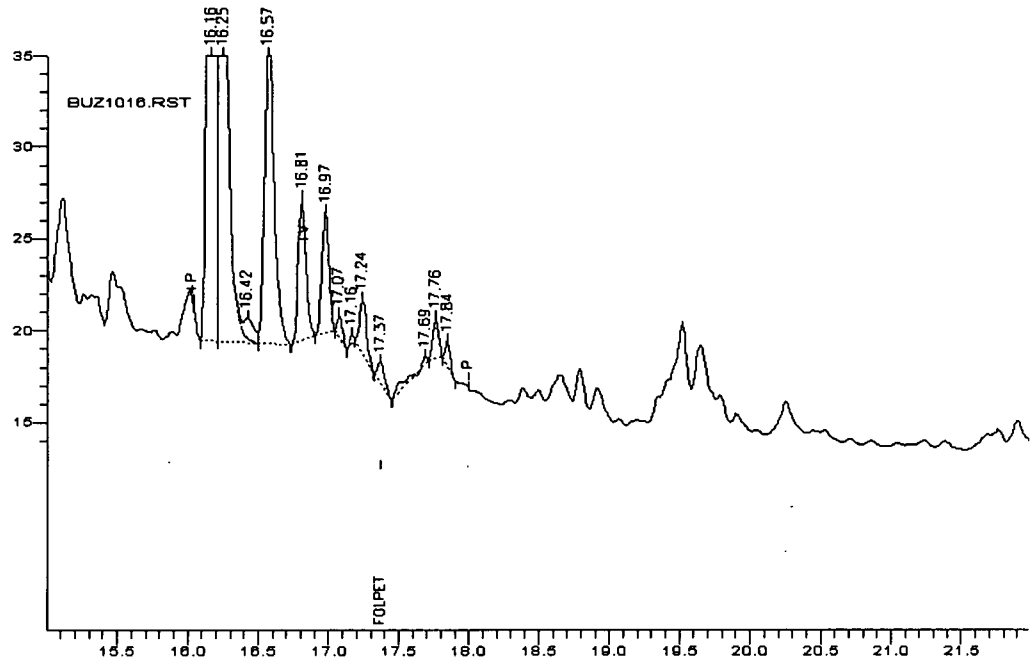

Fig. 3: Chromatogram of an untreated sample of leaf onions

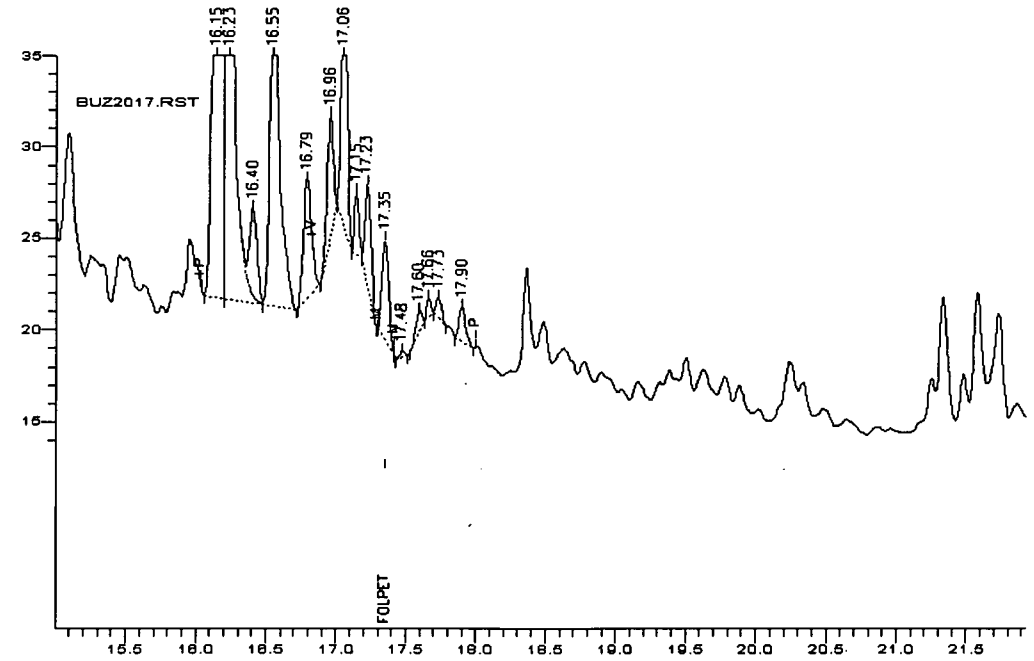

Fig. 4: Chromatogram of an untreated sample of leaf onions fortified with 0.02 mg/kg folpet

Dr. Ehrenstorfer GmbH

D-86199Augsburg

Certified
20 JUNI 1996
by Dr. Heidrich

CERTIFICATE OF ANALYSIS

Product Identification

Common name: Folpet (BSI,ANSI) Code No.:C 138900
Other names: Folpel (France) Lot No:60607
Expiry(at +4°C):06/98
Systematic name: 2-[(trichloromethyl)thio]-1H-isoindole-1,3(2H)-dione (CA)

Formula: $C_9H_4Cl_3NO_2S$ Molecular weight: 296.56

CAS No.: 133-07-3 Use: Fungicide

Purity of the sample was determined by :
O GC X HPLC O TLC X others: Karl-Fischer-Titration: 0.1% water

Elemental analysis calc.:
found:

Documented value of purity: 99 %
(declaration is sensible only to a full percentage due to the variability of purity determinations)

Physical Data

Melting point: 178-180°C decomp. Vapour pressure: < $1,3 \times 10^{-9}$ mbar at 20°C

Solubility: practically insoluble in water, in chloroform 8,7, benzene 2,2, isopropanol 1,25 (all in g/100ml at 20°C)

Product is X stable at 20°C O instable
O hygroscopic O light sensitive
O flammable X moisture sensitive

Toxicological Data

LD_{50}: acute oral dosis for rats 10000 mg/kg

Product is O caustic X skin irritant O toxic
O carcinogen O mutagen
O teratogen X harmful

Special Remarks

Avoid inhalation of dust and keep away from eyes.

28 SEP 1996

The information herein is believed to be correct, but is provided without warranty of any kind.

Fig. 5: Certificate of analysis for folpet reference substance

ARBEITSGEMEINSCHAFT GAB IFU

Fig. 6: GLP Certificate of testing facility

Umweltministerium Baden-Württemberg

GLP-Bescheinigung

Bescheinigung	Certificate
Hiermit wird bestätigt, daß die Prüfungseinrichtung(en)	It is hereby certified that the test facility(ies)
in 75223 Niefern-Öschelbronn, Eutinger Str. 24 und 75173 Pforzheim, Bleichstr. 19	in 75223 Niefern-Öschelbronn, Eutinger Str. 24 and 75173 Pforzheim, Bleichstr. 19
der Arbeitsgemeinschaft GAB/IFU GmbH mit Sitz in D-75223 Niefern-Öschelbronn	of Arbeitsgemeinschaft GAB/IFU GmbH, in D-75223 Niefern-Öschelbronn
am 11.07.1994 und 07./08.02.1995	on 11.07.1994 and 07./08.02.1995
von der für die Überwachung zuständigen Behörde über die Einhaltung der Grundsätze der Guten Laborpraxis inspiziert worden ist (sind).	was (were) inspected by the competent authority regarding compliance with the Principles of Good Laboratory Practice.
Es wird hiermit bestätigt, daß folgende Prüfungen in dieser Prüfeinrichtung nach den Grundsätzen der Guten Laborpraxis durchgeführt werden.	It is hereby certified that studies in this test facility are conducted in compliance with the Principles of Good Laboratory Practice.

Stuttgart, den 08.03.1995

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Prüfungskategorie 1	**Prüfungen auf physikalisch-chemische Eigenschaften und Gehaltsbestimmungen**
Prüfungskategorie 4	**Umwelttoxikologische Prüfungen zu Auswirkungen auf aquatische und terrestrische Organismen**
Prüfungskategorie 5	**Prüfungen zum Verhalten im Boden, Wasser und in der Luft; Bioakkumulation; Metabolismus**
Prüfungskategorie 6	**Prüfungen auf Rückstände**

Dies entspricht den Kategorien nach der Allgemeinen Verwaltungsvorschrift zum Verfahren der behördlichen Überwachung der Einhaltung der Guten Laborpraxis (ChemVwV-GLP) vom 29. Oktober 1990:

"Physikalisch-chemische Eigenschaften und Gehaltsbestimmungen"

"Ökotoxikologische Eigenschaften"

"Verhalten im Boden, Wasser und in der Luft"

"Rückstände"

Stuttgart, den 08.03.1995

Dr. Albrecht

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