AR226 - 1225

# TELOMER RESEARCH PROGRAM

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DAIKIN ASAHI GLASS COMPANY Clariant DUPONT



February 14, 2003

Mary F. Dominiak
U.S. Environmental Protection Agency
EPA East (Mail Code 7405M)
1200 Pennsylvania Avenue NW
Washington, DC 20460
E-mail: dominiak.mary@epa.gov

Dear Mary:

The Telomer Research Program (TRP) and its member companies, Asahi Glass, Clariant, Daikin, and DuPont, are providing to U.S. EPA Docket# AR226 final reports on the acute hazard of 8-2 Telomer B Alcohol (3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluorodecan-1-ol; CAS No. 678-39-7) when tested with standard freshwater aquatic test species. These reports present the results of the acute aquatic toxicity testing conducted by an independent laboratory at the request of the TRP. These studies were conducted according to existing OECD test guidelines and in accordance with the provisions of the appropriate Good Laboratory Practices (GLP) regulations.

The physical-chemical characteristics of the test substance indicate that the test substance is a "difficult" substance, as indicated in OECD Monograph 23 (Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures, OECD 2000), and the experimental designs selected for the acute aquatic toxicity testing were reflective of this observation. Common practices for testing these types of materials, and for evaluating the results from tests of these types of materials, were used during the conduct of the studies and the preparation of the study reports.

The TRP and its member companies believe that the data gathered from these studies are consistent with the hypothesis that the 8-2 Telomer B Alcohol is of low to medium concern in terms of its potential for causing acute effects on aquatic species. If there are questions concerning the technical aspects of the studies submitted, please contact me so that I may direct your questions to the appropriate TRP member company personnel.

Sincerely,

Katie D. Smythe
TRP Administrator

**This document does not contain TSCA CBI.**

- Report -

# 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro-

## Fish (Zebrafish), Acute Toxicity Test, Static, 96 h, Limit Test with the Saturated Solution

acc. to OECD-Guideline No. 203 for Testing of Chemicals (1992)

**Sponsor**
Telomer Research Project
ASAHI GLASS
ATOFINA
CLARIANT
DAIKIN INDUSTRIES LTD
DUPONT

**Author**
Dirk Scheerbaum

**Testing Facility**
DR.U.NOACK-LABORATORIUM
FÜR ANGEWANDTE BIOLOGIE
Käthe-Paulus-Str. 1
D-31157 Sarstedt

**Laboratory Project ID**

| | |
|---|---|
| Project-No. | 020109CK |
| Study-No. | FAZ84111 |



Study Completed August 07, 2002

Original 7 of 7

# Statement of GLP Compliance

| | |
|---|---|
| Title | 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro-<br>Fish (Zebrafish), Acute Toxicity Test, Static, 96 h, Limit Test<br>With the Saturated Solution |
| Guideline | OECD-Guideline for Testing of Chemicals No. 203 |
| Test Item | 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro-<br>(Batch number: P00/001) |
| Test Facility | DR.U.NOACK-LABORATORIUM FÜR ANGEWANDTE BIOLOGIE<br>Käthe-Paulus-Str.1, D-31157 Sarstedt<br>Phone: (+49) 050 66 / 706 70, Fax: (+49) 050 66 / 706 789<br>E-mail: info@noack-lab.de |
| Deviations from GLP Principles | None |

We declare that this study was conducted and reported in compliance with the actual OECD principles of Good Laboratory Practice and the national GLP regulations as specified in the law in force, except deviations mentioned above.

August 07, 2002 .....
(Dirk Scheerbaum, Study Director)

August 07, 2002 .....
(Thomas Geffke, Scientist)

August 07, 2002 .....
(Dr. Udo Noack, Head of Testing Facility)

# Statement of the Quality Assurance Unit

| | |
|---|---|
| Title | 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro-<br>Fish (Zebrafish), Acute Toxicity Test, Static, 96 h, Limit Test<br>With the Saturated Solution |
| Guideline | OECD-Guideline for Testing of Chemicals No. 203 |
| Test Item | 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro-<br>(Batch number: P00/001) |
| Study Director | Dirk Scheerbaum |

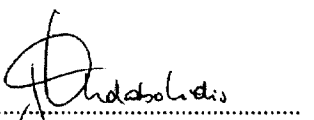
The study was verified as follows:

| inspection | dates | date of report |
|---|---|---|
| study plan | 2002-03-12 | 2002-03-12 |
| process based | 2002-04-02 | 2002-04-02 |
| report | 2002-04-08<br>2002-04-11<br>2002-04-12<br>2002-07-15 | 2002-04-08<br>2002-04-11<br>2002-04-12<br>2002-07-15 |

The reported results accurately and completely reflect the raw data of the study. Also methods, procedures, and observations are accurately and completely described in the report.

The accordance of the study with its study plan and the principles of Good Laboratory Practice is guaranteed.

August 07, 2002



Gudrun Möhrmann-Kalabokidis

# Personnel Involved

| | |
|---|---|
| Study Director: | Dirk Scheerbaum<br>(Biologist) |
| Scientist: | Thomas Geffke<br>(Chemical Engineer)<br>Responsible for concentration control analysis |
| Technical Staff: | Christian Rebl |
| Quality Assurance Unit: | Gudrun Möhrmann-Kalabokidis<br>(Biologist) |
| Deputy: | Susanne Becker<br>(Biologist) |
| Director of the Testing Facility: | Dr. Udo Noack<br>(Biologist) |

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# 1 Summary

The acute toxicity of the test item 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro- (Batch no.: P00/001) to fish (zebrafish) was determined according to the OECD-Guideline for Testing of Chemicals No. 203 (1992) from 2002-04-02 to 2002-04-06 at DR.U.NOACK-LABORATORIUM FÜR ANGEWANDTE BIOLOGIE, 31157 Sarstedt, Germany.

A static limit test was conducted with the saturated solution. The test vessel was coated with the test item for 14 days before the test started. Duration of the test was 96 h. 30 test organisms were exposed to the saturated solution and the control. For the preparation of the saturated solution a suspension with a nominal loading of 100 mg/L was shaken for 96 h and centrifuged. The clear supernatant was used for the test.

All effect values are based on the initially measured concentration of the test item. GC-MS analysis was carried out from the test item and the control vessel at test start and end (for results see part 9.2). The initially measured concentration of 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro- was 0.18 mg/L. At the end of the test the concentration of the test item was 0.0063 mg/L. Sorption to the glass walls of the test vessels is unlikely as the walls were already pre-coated for 14 d before the test has started.

The acute toxicity of the test item 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8, 9,9,10,10,10-heptadecafluoro- is summarised in the following table:

Tab. 1: **LC-Values with 95 % Confidence Intervals**
(based on initially measured concentrations)

| Test duration [h] | $LC_{50}$ [mg/L] | p = 95 % [mg/L] |
|---|---|---|
| 24 | > 0.18 mg/L | not applicable |
| 48 | > 0.18 mg/L | not applicable |
| 72 | > 0.18 mg/L | not applicable |
| 96 | > 0.18 mg/L | not applicable |
| $LC_{100}$ = Lowest test item concentration with 100 % mortality after 96 h | | > 0.18 mg/L |
| $LC_0$ = Highest test item concentration with 0 % mortality after 96 h | | 0.18 |
| NOEC (0-96 h) = No observed effect concentration | | 0.18 |

Water quality parameters of pH-value and oxygen-saturation measured after 0, 24, 48, 72 and 96 h were determined to be within the acceptable limits.

## 2 Characterisation Data of the Test Item

| | |
|---|---|
| TEST ITEM | 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro- |
| Batch Number | P00/001 |
| CAS RN | 678-39-7 |
| Chemical characterization | 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluorodecane-1-ol (EINECS) |
| Purity | C8-perfluoroalkylethanol: 99.3 % (a/a),<br>sum perfluoroalkylethanol 99.9 % (a/a) |
| Appearance | Yellowish solid wax |
| Water solubility | 134 µg/L at 12 degree C, Std 29 µg/L, n=8 (DuPont, 2002)<br>137 µg/L at 25 degree C, Std 53 µg/L, n=17 (DuPont, 2002) |
| Stability in water | Stable at test conditions |
| Density | Ca. 1.7 g/cm$^3$ |

| | |
|---|---|
| Expiry date | Unlimited if stored under the recommended conditions. |
| Recommended storage | Room temperature (20 °C), protected from moisture. |
| Storage at testing facility | Room temperature, protected from moisture and light. |
| Retention | At least 1 g has been retained. |
| Identification parameter at testing facility | Name, batch number and colour. |

*The test item and the information concerning the test item were provided by the sponsor.*

# 3 Method

| | |
|---|---|
| **TEST GUIDELINE** | OECD-Guideline for Testing of Chemicals No. 203 (1992) |
| **TYPE AND PURPOSE** | Determination of the acute toxicity of 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro- to zebrafish in a limit test with the saturated solution. |
| **TEST SYSTEM** | *Danio rerio* (zebrafish)<br>Gnathostoma, Pisces, Osteichthyes, Teleostei, Clupeiformes, Cyprinidae |
| Reason for the selection of the test system | According to the guideline *Danio rerio* is suitable for this kind of study. |
| Origin | AQUARIUM AM AEGI, Feldstr. 7, D-30159 Hannover<br>All fish used in the test will originate from the same delivery of the supplier. |
| Holding | Holding was performed at the test facility at 23 ± 2 °C and diffuse light (0.1-10 µE/m$^2$s, natural photoperiod). The water was changed at least once per week. No disease treatment was administered.<br>The dissolved oxygen concentration was more than 80 % of the air saturation value.<br>Only zebrafish with at least 12 days of acclimatisation and mortality < 5 % within the last 7 days before the study starts was used in the test. |
| Water | Tap water of local origin was used for holding and testing. The water was filtered on activated charcoal and aerated for at least 24 h to remove chlorine.<br>Nominal water parameters:<br>Total hardness: 40 - 180 mg $CaCO_3$/L<br>pH-value: 6.0 - 8.0<br>The water is analysed once a year acc. to German tap water regulation. |
| Feeding | Food was provided 3 times per week. The amount of food was 4 % of the fish body weight per feeding day. The test fish will not be fed until 24 h before the test started. |
| Food | Trouvit 40 / 2; MILKIVIT, D-86666 Bürgheim |

| | |
|---|---|
| **TEST ITEM** | **1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro-** |
| Pretreatment of | The test item was heated to 50 °C before weighing for homogenisation. |
| **TEST CONDITIONS** | |
| **Coating of the Test vessels** | A coating phase of 14 days was carried out. |
| Preparation | 100 mg 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro- /L dilution water (total volume 5 x 2 L) were shaken in a rotating shaker with 20 rpm for 96 h at 20 °C. 2 L – brown glass flasks were used. After phase separation about 10 L per test replicate were transferred from the clear liquid phase to the glass aquarium and covered with glass tops. Dilution water was used for the preparation of the coating solution. |
| Coating period | The readily filled and closed aquarium was stored at nominally 23 ± 2 °C for 14 days. At the end of the coating period the aquarium was emptied and used for the test. |
| Preparation of the test medium (saturated solution) | 100 mg 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro- /L dilution water (total volume 5 x 2 L) were shaken in a rotating shaker with 20 rpm for 96 h at 20 °C. 2 L –brown glass flasks were used. This suspension was centrifuged for 20 min at 3000 rpm. 10 L per test replicate of the clear saturated solution was transferred to the glass aquarium. The preparation method has been chosen with regard to the test item properties and in accordance with the relevant guidance documents (OECD 2000, BAUA 2001). |
| **CONTROL** | 30 fish in dilution water (without test item) were tested under the same test conditions as the test replicates. |
| **REFERENCE ITEM** | No reference item is recommended for this test according to the guideline. |
| **TEST METHOD** | A static limit test was performed with one application at the test initiation. |

| | |
|---|---|
| Test duration | 96 h |
| Test vessel | Glass-aquaria loosely covered by glass tops were used. |
| Test volume | The test volume was 10 L per vessel. |
| Aeration | Gentle aeration was provided. |
| Dilution water | Same as for holding. |
| Number of test fish | 30 zebrafish was used per test concentration and control. |
| Loading, length of fish | Fish density in the tanks was less than 1 g fish per liter test solution.<br>Average body length at the test start: 2.77 cm<br>Average body weight at the test start: 0.29 g<br>Measuring values were determined with fish from the same batch but not used in the test. |
| Replicates | One replicate per test concentration and control. |
| Introduction of fish | Fish were introduced randomly to individual replicates. |
| Feeding of test fish | The fish will not be fed during the test. |
| Water temperature | 23 ± 2 °C |
| Dissolved oxygen concentration | Not less than 60 % of air saturation value. |
| Light intensity | 0.1 - 10 µE / $m^2 \cdot s$ |
| Photoperiod | A natural photoperiod occurred during the test. |

**KIND AND FREQUENCY OF MEASUREMENT AND OBSERVATIONS**

| | |
|---|---|
| Water quality measurements | pH-value, temperature and oxygen saturation were measured in all vessels at the beginning of the test and every 24 h.<br>Total hardness of the water was determined at the beginning out of the test control. During the test the room temperature was recorded continuously with a thermohygrograph. |

| | |
|---|---|
| Criteria of effects | Fish were considered dead if there was no visible movement (e.g. gill covers movement) and if touching of the caudal peduncle produced no reaction. Records were kept of visible abnormalities (e.g. loss of equilibrium, swimming behaviour, respiratory function, pigmentation, etc.). |
| Observation intervals | Observations were made after 24, 48, 72 and 96 h. |
| Measurement of fish size | At test start body weight and total length of a representative number of 7 test fish was determined. These fish will not be used for testing. |
| **DETERMINATION OF THE TEST ITEM** | The test concentration level and control were analytically verified by gas chromatography with mass spectrometric detection (m/z = 463 and 395) at test start and test end. For details see part 9.1. |
| Sampling and storage | 40 mL test solution were transferred to 50 mL glass flasks closed with glass plugs and stored at 2 - 7 ° in the dark until analysis (test start for 3, test end for 2 days). |
| **EVALUATION** | The concentrations leading to 0 and 100 % mortality ($LC_0$ and $LC_{100}$) as well as the no effect level (NOEC) after 96 h were determined directly from the test results.<br>$LC_{50}$-values have not to be calculated in a limit test. |
| **COURSE OF THE STUDY** | The definitive study was performed as a limit test with the saturated solution as single test concentration.<br><br>One control with water (without test item) was tested under the same conditions as the test group.<br><br>In the test concentration and control 30 fish were tested.<br>The test fish were not fed during the test.<br><br>After 24, 48, 72 and 96 h mortality and behaviour of the test fish were observed.<br><br>Measurements and observations were carried out as described above. |

**VALIDITY CRITERIA**

- $O_2$ - saturation ≥ 60 %
- Mortality in the control ≤ 10 %

**DEVIATIONS FROM THE GUIDELINE**

None

**DEVIATIONS FROM THE STUDY PLAN**

None

**REPORTING**

The final report is issued as paper copy (6 originals) and an electronic record (1 original).

**ARCHIVING**

The following was retained in the archive of the test facility for the period as specified in the operative national GLP regulations:

- all raw data
- study plan (Original 1 of 2)
- final report paper copy (Original 1 of 6)
- all records performed by the quality assurance programme including master schedules
- sample of test item

Microfilms were retained in a safe-deposit by Volksbank Sarstedt, D-31157 Sarstedt.

# 4 Results

## 4.1 Preliminary Test

Tab. 2: **Cumulative Mortality in [%] in the Preliminary Test**
(n = 5)

| Concentration | Effect*) | Test Duration [h] | | | |
|---|---|---|---|---|---|
| | | 24 | 48 | 72 | 96 |
| Saturated Solution | (1) | 0 | 0 | 0 | 0 |
| Control | (1) | 0 | 0 | 0 | 0 |

*) The number in brackets correspond to the following observation:
(1) = Normal behaviour

## 4.2 Definitive Test

Symptoms of toxicity during the test are given in table 3. The cumulative mortality at each time of observation is shown in table 4.

The test item concentration level caused no mortality. Therefore the NOEC was laid down as 0.18 mg/L (based on the initially measured concentration).

Tab. 3 **Observations in the Test Vessels**

| Initially Measured Concentration | Effect*) | Test Duration [h] | | | |
|---|---|---|---|---|---|
| | | 24 | 48 | 72 | 96 |
| 0.18 mg/L | (1) | 30/30 | 30/30 | 30/30 | 30/30 |
| Control | (1) | 30/30 | 30/30 | 30/30 | 29/30 |
| | (E) | - / - | - / - | - / - | 1/30 |

*) The number in brackets correspond to the following observation:
(1) = Normal behaviour (E) = Exitus letalis (Mortality)

Tab. 4: **Cumulative Mortality in [%] in the Test Vessels**

| Initially Measured Concentration | Test Duration [h] | | | |
|---|---|---|---|---|
| | 24 | 48 | 72 | 96 |
| 0.18 mg/L | 0 | 0 | 0 | 0 |
| Control | 0 | 0 | 0 | 3 |

Tab. 5: **$LC_0$-, $LC_{100}$-, NOEC-Values (0 - 96 h)**
Based on Initially Measured Concentrations

| | |
|---|---|
| $LC_{100}$ = Lowest test item concentration with 100 % mortality after 96 h | > 0.18 mg/L |
| $LC_0$ = Highest test item concentration with 0 % mortality after 96 h | 0.18 mg/L |
| NOEC (0-96 h) No observed effect concentration | 0.18 mg/L |

Tab. 6: **$LC_{50}$-Values with 95 % Confidence Intervals**
Based on Initially Measured Concentrations

| Test Duration [h] | $LC_{50}$ [mg/L] | p = 95 % [mg/L] |
|---|---|---|
| 24 | > 0.18 mg/L | not applicable |
| 48 | > 0.18 mg/L | not applicable |
| 72 | > 0.18 mg/L | not applicable |
| 96 | > 0.18 mg/L | not applicable |

# 5 Validity Criteria

The study met the validity criteria of the guideline:

- $O_2$ - saturation was ≥ 60 %
- Mortality in the control was 3 %

# 6 Conclusions

In this study 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro- did not cause any effects to zebrafish after 96 h when tested at the initially measured concentration of 0.18 mg/L. The NOEC (0 – 96 h) is 0.18 mg/L (initially measured concentrations).

# 7 Literature / References

(1) OECD-guideline 203 for testing of chemicals. Fish, Acute Toxicity Test. Adopted July 17, 1992

(2) OECD (2000): Guidance document on aquatic toxicity testing of difficult substances and mixtures. OECD series on testing and assessment no. 23

(3) BAUA, Bundesanstalt für Arbeitsschutz und Arbeitsmedizin (2001): Leitfaden für Anmeldungen neuer Stoffe nach dem Chemikaliengesetz. 3. überarbeitete Auflage.

(4) DuPont, Determination of physico-chemical parameters of Perfluorooctylethanol, CAS No. 678-39-7, Unpublished results, Update Feb. 15, 2002

# 8 Physicochemical Data

## 8.1 Water Quality Measurements

Tab. 7: **Water Parameters**

| | Initially measured Concentration | Oxygen-Saturation [%] | pH-Value | Temperature [°C] | Total Hardness [mg/L as $CaCO_3$] |
|---|---|---|---|---|---|
| day 0 | 0.18 mg/L | 100 | 7.42 | 21.5 | |
| | Control | 100 | 7.46 | 21.1 | 71 |
| day 1 | 0.18 mg/L | 94 | 6.98 | 21.9 | |
| | Control | 90 | 6.93 | 21.8 | |
| day 2 | 0.18 mg/L | 94 | 7.43 | 21.0 | |
| | Control | 94 | 7.47 | 21.0 | |
| day 3 | 0.18 mg/L | 94 | 7.31 | 21.0 | |
| | Control | 93 | 7.45 | 21.0 | |
| day 4 | 0.18 mg/L | 90 | 7.77 | 21.1 | |
| | control | 88 | 7.81 | 21.2 | |

# 9 GC – Analysis of 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9, 10,10,10-heptadecafluoro-

## 9.1 Method

METHOD OF DETERMINATION

Analyses of various concentrations of 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro- were carried out via gas chromatography on a Fused Silica CP-SIL 8 capillary column using external standards. Detection was performed with a mass selective detector (MS).

Equipment

| | |
|---|---|
| Gas chromatograph | : CP-3800, VARIAN |
| Autosampler | : CTC Combi PAL, CTC ANALYTICS |
| Detector | : MS, Saturn 2000, VARIAN |
| Software | : Saturn GC/MS Workstation 5.51, VARIAN |

Reagents

*tert.*-butyl methyl ether 99.8 %, Batch 423370/1 31101, FLUKA

CONDITIONS OF ANALYSIS

Column

Chrompack CP-SIL 8 (VARIAN), 30 m, i.d.: 0.25 mm, LOW BLEED / MS, Film thickness: 0.25 µm

Injector

Splitless (30 psi pressure puls) for 1 min

Injector temperature

250 °C

Injection volume

1 µL

Temperature programme

| Heating rate [°C/min] | Temperature [°C] | Holding time [min] |
|---|---|---|
| --- | 40 | 0.0 |
| 10 | 90 | 1.0 |
| 30 | 220 | 5.0 |

Carrier gas

Helium 1 mL / min column flow

Detector

MS

Ionisation mode

Electron ionisation (EI)

Run time

15.33 min

Retention time

approx. 4.9 min

| | |
|---|---|
| STANDARD | Test item was used as standard. |
| PREPARATION OF STANDARDS | Two stock solutions of the standard of 200 mg/L were prepared in *tert.*-butyl methyl ether, diluted (9 concentrations each) and used for calibration. For calibration range see part 9.2.1. |
| PREPARATION OF SAMPLES | 40 mL of the sample were extracted with 4.0 mL *tert.*-butyl methyl ether.<br>The extraction step encloses an enrichment by factor 10.<br>After phase separation the extract was injected directly into the GC/MS. |
| METHOD VALIDATION | Quantification of the test item was calculated by peak area of the masses 69 + 395 + 463 (m/z) based on the external standards. The calibration procedure and the principle for the determination of detection and quantification limits were adapted from DIN 32645. The method was validated as described below: |
| Peak symmetry | Peak symmetries were calculated from chromatograms of analytical standards and test item according to the following equation:<br><br>$T = W_{0.05} / (2 \cdot f)$<br><br>T = Peak symmetry<br>$W_{0.05}$ = Peak width at 5 % of peak height [mm]<br>f = first sector of peak width ( divided by the perpendicular line from the peak maximum ) [mm] |
| Repeatability | 5 sub-samples of the highest and lowest concentration prepared from a single homogenous sample were analysed. Mean values, standard deviations and variations of coefficients were calculated. |
| Accuracy | 5 samples with 40 mL water were spiked with an amount of 8 µg 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro- (80 µL of 100 mg/L) to give a final concentration of 200 µg/L. Afterwards samples were extracted as described above. |

### 9.2 Method Validation

The method was validated and tested with satisfactory results with respect to

- linearity
- precision (repeatability)
- peak symmetry
- accuracy
- determination limit and detection limit.

#### 9.2.1 Linearity

The analytical system gave linear response in the range of 0.1 - 10 mg/L of 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro-. The calibration curves and chromatograms obtained for standard and test item are given in figures 1 - 6. For determination and detection limits see part 9.3.

#### 9.2.2 Quantification Limit and Detection Limit

The limit of quantification (LOQ) and limit of detection (LOD) were calculated using the blind value method described in DIN 32645 "Nachweis- und Bestimmungsgrenze".
The LOD was determined to be 0.009 mg/L and therefore the LOQ was 0.027 mg/L (threefold the LOD).

### 9.2.3 Precision (Repeatability)

The results of the repeatability tests are provided in the table below.

Tab. 8: **Repeatability of Injections of Test Item**
Peak area [µV*s]

| Serial No | 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro- (m/z = 69+395+463) | |
|---|---|---|
| | 0.1 mg/L | 0.6 mg/L |
| 1 | 2098 | 12141 |
| 2 | 2098 | 13563 |
| 3 | 1978 | 13291 |
| 4 | 1958 | 13597 |
| 5 | 2341 | 13500 |
| Mean ± SD | 2094.6 ± 152.5 | 13218.4 ± 613.9 |
| CV [%] | 7.3 | 4.6 |

### 9.2.4 Peak Symmetry

The peak symmetry of the test item 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro- was 1.8.

### 9.2.5 Accuracy

Tab. 9: **Mean Recovery Rate of Fortified Samples**

| Fortified concentration of test item | 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10 -heptadecafluoro- | |
|---|---|---|
| | calc. conc. | RR |
| 0.1986 [mg/L] | [mg/L] | [%] |
| Replicate 1 | 0.22 | 111 |
| | 0.24 | 121 |
| Replicate 2 | 0.23 | 116 |
| | 0.21 | 106 |
| Replicate 3 | 0.21 | 106 |
| | 0.21 | 106 |
| Replicate 4 | 0.22 | 111 |
| | 0.21 | 106 |
| Replicate 5 | 0.21 | 106 |
| | 0.21 | 106 |
| Mean | | 110 |
| SD (N-1) | | 5.3 |
| CV % | | 4.8 |
| LOQ [mg/L] | 0.027 | |
| LOD [mg/L] | 0.009 | |
| Linearity correlation coefficient ($r^2$) | 0.9906 | |

calc. conc. = calculated concentration, accumulation factor taken into account
RR = Recovery rate related to the nominal concentration of the test item
LOQ = Limit of quantification
LOD = Limit of detection
SD (N-1) = standard deviation
CV = coefficient of variation

## 9.3 Results

Tab. 10: **Concentrations of the Active Ingredient 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10, 10,10-heptadecafluoro-**

| Sampling Date | | 2002-04-02 Test Start | 2002-04-06 Test End | |
|---|---|---|---|---|
| Start of Analysis | | 2002-04-05 | 2002-04-08 | |
| Test Item | Replicate No. | 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro- | | |
| | | Calc. Conc. [mg/L] | Calc. Conc. [mg/L] | Mean[1] [mg/L] |
| Saturated solution | 1 | 0.14 | 0.0063[2] | 0.18 |
| | | 0.16 | 0.0064[2] | |
| | 2 | 0.21 | 0.0062[2] | |
| | | 0.19 | 0.0063[2] | |
| Control | | < LOQ | < LOQ | |
| | | < LOQ | < LOQ | |
| LOQ [mg/L] | | 0.027 | | |
| LOD [mg/L] | | 0.009 | | |
| linearity ( $r^2$ ) | | 0.9996 | | |

calc. conc. = calculated concentration, accumulation factor taken into account
LOQ = Limit of quantitation
LOD = Limit of detection
1) = concentrations from the test end were not taken into account for mean value
2) = see explanation below

Explanation of data reporting
The concentrations calculated at the test end are based on 10fold enriched samples. As the concentrations in the enriched samples were all above the LOQ of 0.027 mg/L (e.g. 0.063 mg/L) the sample concentrations in the test can be calculated dividing these concentrations by 10. This delivers figures (e.g. 0.0063 mg/L) which are below the LOD and have to be reported as calculated figures under GLP. These calculated values are no artefacts. If the concentration in the enriched samples would have been below the LOQ (e.g. 0.027 mg/L) a division by 10 would not be allowed and the test concentration at the end of the test would be given as < LOQ.

### 9.4 Calibration Curves

The calibration curves shown were established for different concentration ranges and at different times

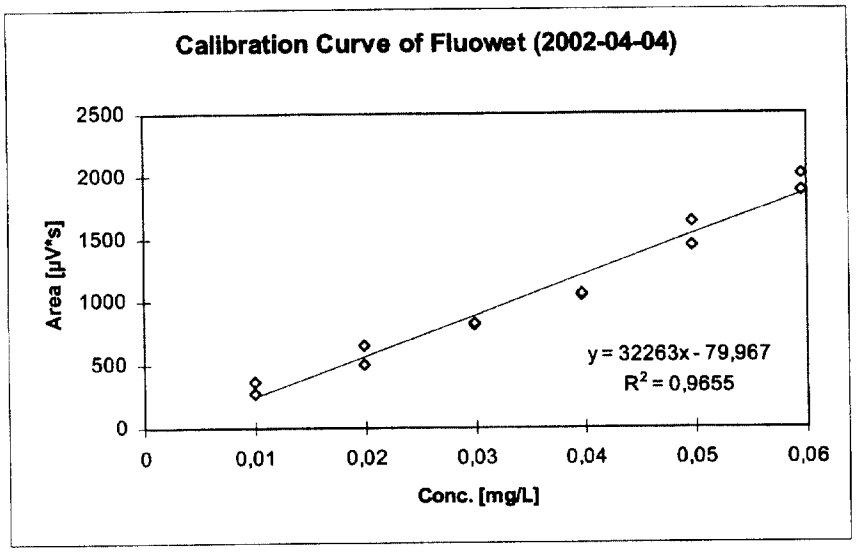



Fig. 1: **Calibration Curve of Standard - concentration range 0.01 - 0.06 mg/L** (dated 2002-04-04)

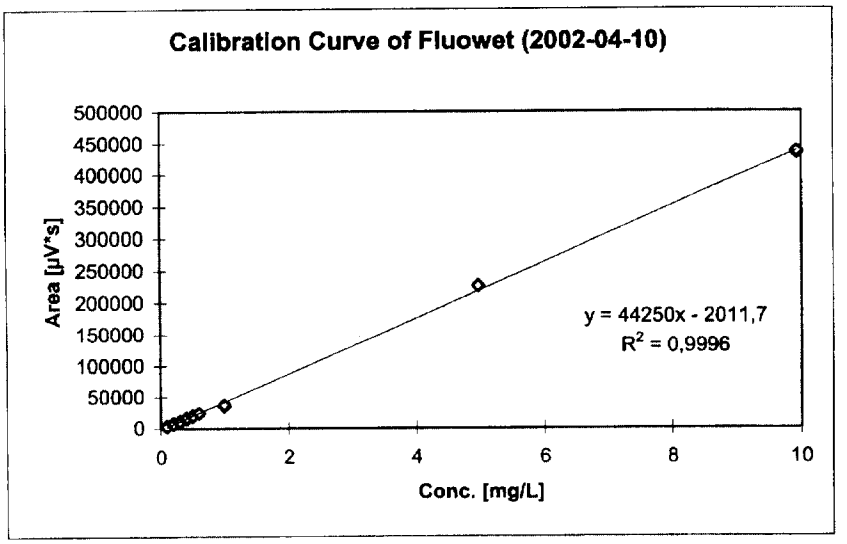



Fig. 2: **Calibration Curve of Standard - concentration range 0.1 - 10 mg/L** (dated 2002-04-10)

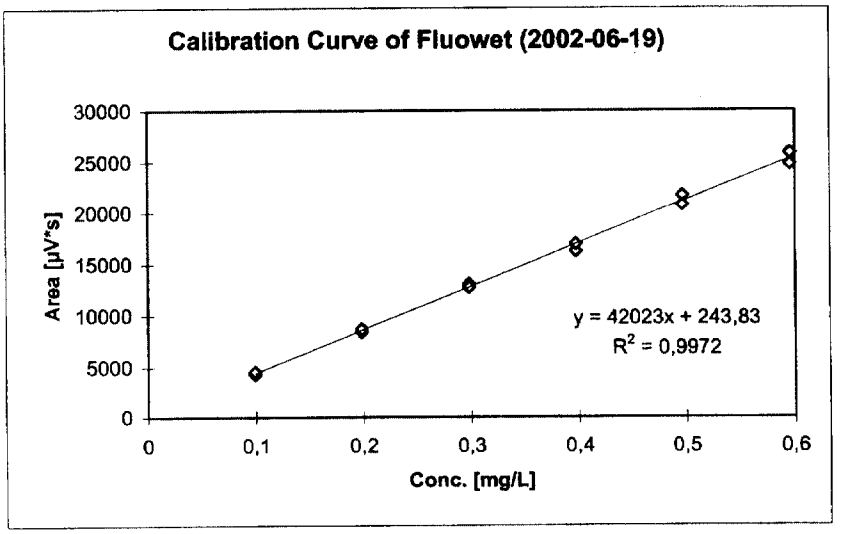



Fig. 3: **Calibration Curve of Standard - concentration range 0.1 - 0.6 mg/L** (dated 2002-06-19)

The first calibration curve was established 2002-04-04 but the concentration range (0.01 - 0.06 mg/L) was not broad enough to be used for all tests running. Therefore the second curve (2002-04-10) which covers a much wider range (0.1 - 10 mg/L) was used for the calculations in this test. The concentration calculations using the two different curves do not differ more than 10 % from each other.
The calibration curve from 2002-06-19 with a concentration range of 0.1 - 0.6 mg/L shows that the GC/MS system has a good stability over a long period of time.

## 9.5 Chromatograms

Target Compound Report for #1 from 8411 0.1 l_1.SMS

| | | | |
|---|---|---|---|
| Sample ID: | 8411 0.1 l | Operator: | |
| Instrument ID: | | Last Calibration: | 11.04.02 08:08 |
| Measurement Type: | Area | Calibration Type: | External Standard |
| Acquisition Date: | 10.04.02 13:21 | Data File: | c:\... 8411 0.1 i_1.sm |
| Calculation Date: | 11.04.02 08:08 | Method: | c:\... \8411_rep2.mth |
| Sample Type: | Calibration | | |
| Inj. Sample Notes: | | | |
| Divisor: | 1,000000 | Multiplier: | 1,000000 |

| Parameter | Specification | Actual | Status |
|---|---|---|---|
| Compound Number | | 1 | |
| Retention Time | 4,918 +/- 0,200 | 4,911 min. | Pass |
| RT Offset | | Δ0,00676 min. | |
| Peak Name | | Fluowet | |
| CAS Number | | 678-39-7 | |
| Status | | | |
| Quan Ions | 69+395+463 | | |
| Area | >=10 | 4368 | Pass |
| Height | | 2283 | |
| Amount (RF) | | 44124,941 | |
| Fit | >=700 | 971 | Pass |
| Calibration Equation | Linear, Include, None | y = +4,4125e+4x +0,0 | |
| Baseline Code | | BB | |

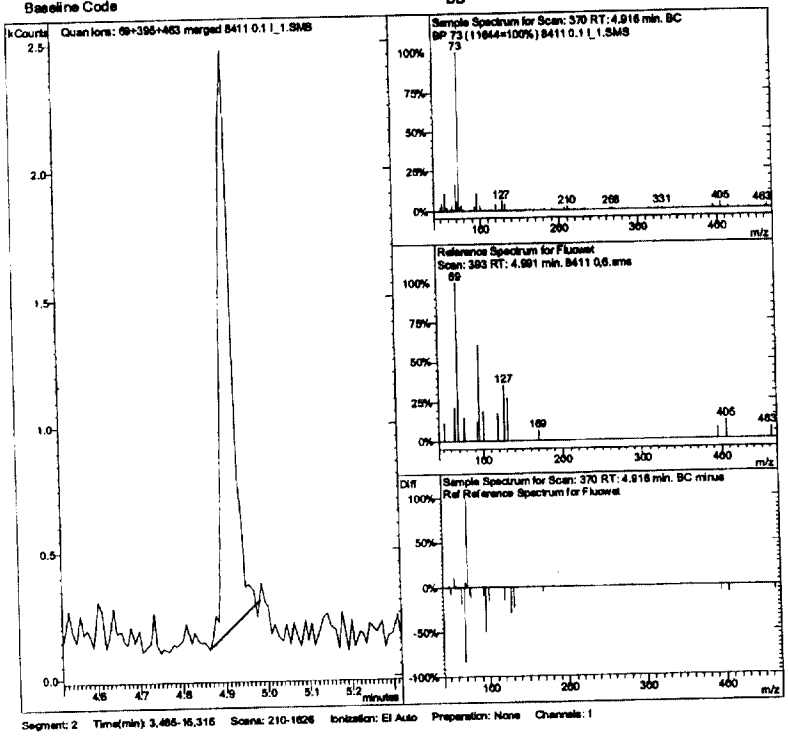
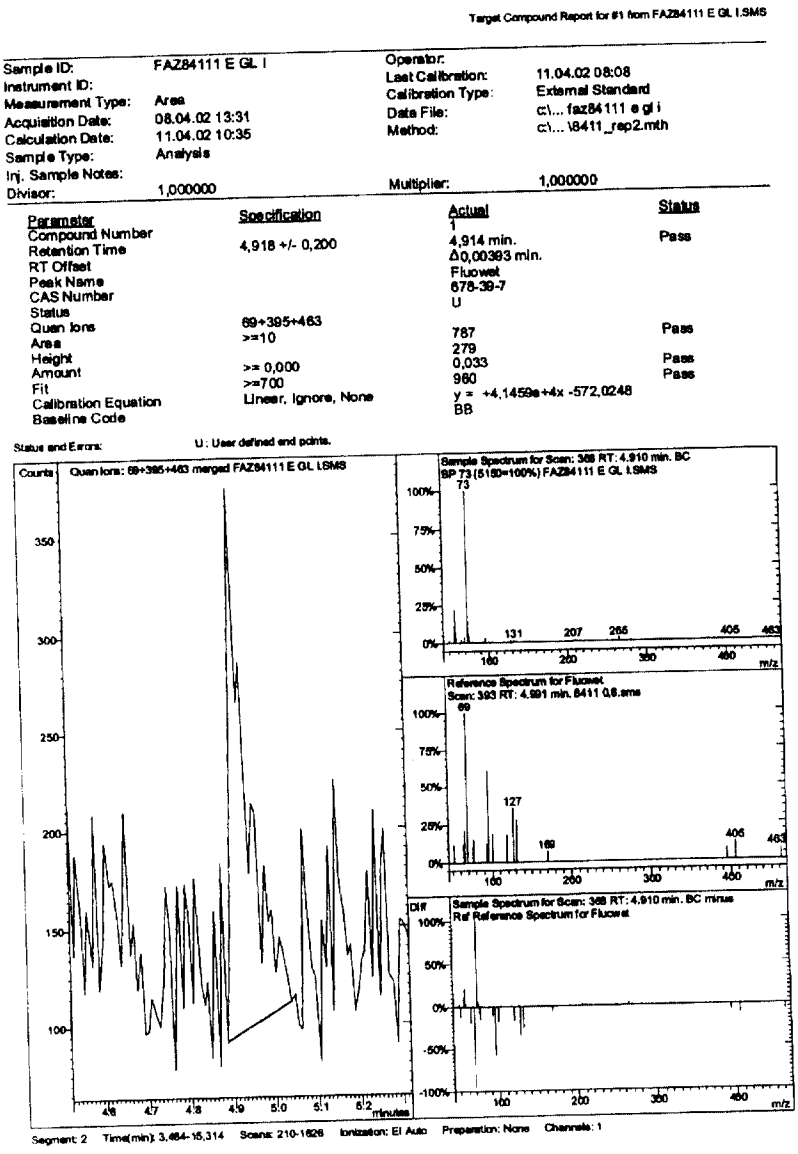



Fig. 4: **Chromatogram of Standard**
0.1 mg/L (dated 2002-04-10)



Target Compound Report for #1 from FAZ84111 E GL I.SMS

| | | | |
|---|---|---|---|
| Sample ID: | FAZ84111 E GL I | Operator: | |
| Instrument ID: | | Last Calibration: | 11.04.02 08:08 |
| Measurement Type: | Area | Calibration Type: | External Standard |
| Acquisition Date: | 08.04.02 13:31 | Data File: | c:\... faz84111 e gl i |
| Calculation Date: | 11.04.02 10:35 | Method: | c:\... \8411_rep2.mth |
| Sample Type: | Analysis | | |
| Inj. Sample Notes: | | | |
| Divisor: | 1,000000 | Multiplier: | 1,000000 |

| Parameter | Specification | Actual | Status |
|---|---|---|---|
| Compound Number | | 1 | |
| Retention Time | 4,918 +/- 0,200 | 4,914 min. | Pass |
| RT Offset | | Δ0,00393 min. | |
| Peak Name | | Fluowet | |
| CAS Number | | 678-39-7 | |
| Status | | U | |
| Quan Ions | 69+395+463 | | |
| Area | >=10 | 787 | Pass |
| Height | | 279 | |
| Amount | >= 0,000 | 0,033 | Pass |
| Fit | >=700 | 960 | Pass |
| Calibration Equation | Linear, Ignore, None | y = +4,1459e+4x -572,0248 | |
| Baseline Code | | BB | |

Status and Errors: U : User defined end points.



Segment: 2 Time(min): 3,464- 15,314 Scans: 210-1626 Ionization: EI Auto Preparation: None Channels: 1

Fig. 5: **Chromatogram of Test Item**
Saturated solution at the test end (dated 2002-04-08)

Target Compound Report for #1 from FAZ84111_2 E Klr.SMS

| | | | |
|---|---|---|---|
| Sample ID: | FAZ84111_2 E Klr | Operator: | |
| Instrument ID: | | Last Calibration: | 11.04.02 08:08 |
| Measurement Type: | Area | Calibration Type: | External Standard |
| Acquisition Date: | 08.04.02 12:49 | Data File: | c:\... faz84111_2 e kl |
| Calculation Date: | 11.04.02 10:31 | Method: | c:\... \8411_rep2.mth |
| Sample Type: | Analysis | | |
| Inj. Sample Notes: | | | |
| Divisor: | 1,000000 | Multiplier: | 1,000000 |

| Parameter | Specification | Actual | Status |
|---|---|---|---|
| Compound Number | | 1 | |
| Retention Time | 4,918 +/- 0,200 | 5,073 min. | Pass |
| RT Offset | | Δ0,15453 min. | |
| Peak Name | | Fluowet | |
| CAS Number | | 678-39-7 | |
| Status | | M | |
| Quan Ions | 69+395+463 | | |
| Area | >=10 | 0 | Fail |
| Height | | 0 | |
| Amount | >= 0,000 | 0,000 | Pass |
| Fit | >=700 | 473 | Fail |
| Calibration Equation | Linear, Ignore, None | y = +4,1458e+4x -572,0248 | |
| Baseline Code | | | |

Status and Errors: M: Missing peak.

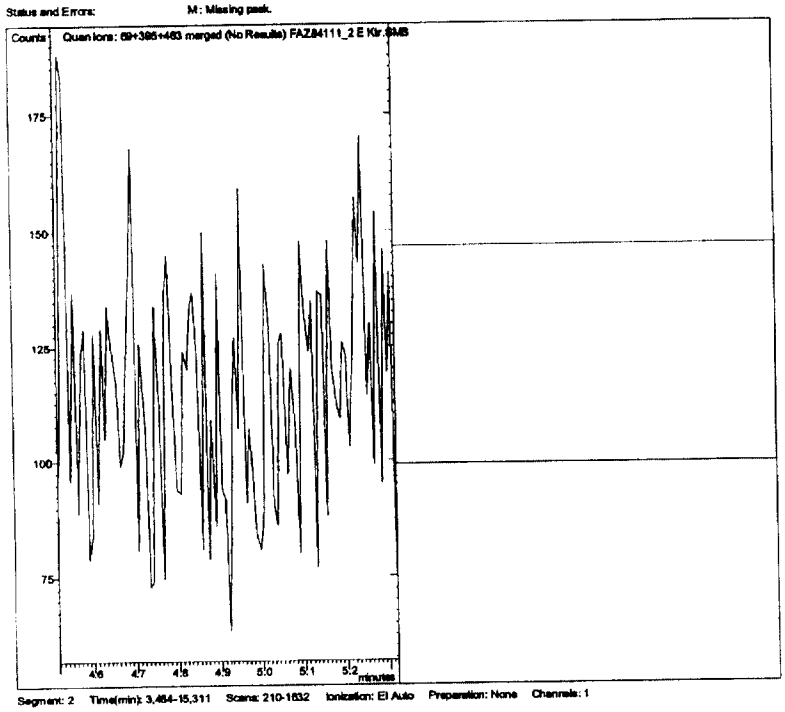



Fig. 6: **Chromatogram of Control**
at test end (dated 2002-04-08)

# 10 Certificate of 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro- Analysis

Clariant GmbH, Werk Gendorf
CQG / Qualitätsmanagement
D-84504 Burgkirchen
Fax: ++49 8679 / 2676

Clariant

Inspection certificate
according to EN10204-3.1B

Date: 13.11.2001
Page: 1 / 1

Our consignment
Material : 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro
Material-no. : 104705
Batch No. : P00/001

On the batch, of which the consignment is a part, the following values were determined. They conform to the agreed product specification

| Inspection characteristic/-method | Specification | Result |
|---|---|---|
| C8-Perfluoralkylethanol Capillary GC | >= 97,5 | 99,3%[a/a] |
| Sum Perfluoroalkylethanol Capillary GC | >= 98,0 | 99,9%[a/a] |
| Sum Perfluoralkylbutanol Capillary GC | <=1,0 | < 0,1%[a/a] |
| Sum Perfluoralkylethene Capillary GC | <=0,5 | < 0,1%[a/a] |
| Water content Karl Fischer DIN 51777 | <=0,2 | < 0,1% |
| N-Methylpyrrolidone content Capillary GC | <=0,2 | < 0,1% |

The above particulars do not release the customer from the obligation to carry out an inspection of goods received.

Ms. Baumgartner - Worksinspector

This report is not to be signed

# 11 GLP-Certificate of DR.U.NOACK-LABORATORIUM

Niedersächsisches Umweltministerium

Gute Laborpraxis/Good Laboratory Practice

## GLP-Bescheinigung/Statement of GLP Compliance

(gemäß/according to § 19 b Abs.1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 88/320/EG wurde durchgeführt in:

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 88/320/EEC at:

X Prüfeinrichtung / Test facility ☐ Prüfstandort / Test site

**Dr. U. NOACK LABORATORIUM**
für angewandte Biologie
Käthe-Paulus-Str. 1
D-31157 Sarstedt

**Dr. U. NOACK LABORATORY**
of applied biology
Käthe-Paulus-Str. 1
D-31157 Sarstedt

(Unverwechselbare Bezeichnung und Adresse/Unequivocal name and address)

Prüfungen nach Kategorien/Areas of Expertise
(gemäß/according ChemVwV-GLP Nr. 5.3/OECD guidance)

1 - Prüfungen zur Bestimmung der physikalisch-chemischen Eigenschaften und Gehaltsbestimmungen
3 - Prüfungen zur Bestimmung der erbgutverändernden Eigenschaften (in vitro und in vivo)
4 - Umwelttoxikologische Prüfungen zu Auswirkungen auf aquatische und terrestrische Organismen
5 - Prüfungen zum Verhalten im Boden, im Wasser und in der Luft, Prüfungen zur Bioakkumulation und zur Metabolisierung
6 - Prüfungen zur Bestimmung von Rückständen
7 - Prüfungen zur Bestimmung der Auswirkungen auf Mesokosmen und natürliche Ökosysteme

Datum der Inspektion / Date of Inspection
(Tag.Monat.Jahr / day.month.year)
**25. - 27. Juni 2001 / June 25th - 27th, 2001**
**29. August und 21. September 2001 / August 29th and September 21st, 2001**

Die genannte Prüfeinrichtung/~~Der genannte Prüfstandort~~ befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

The above mentioned test facility / test-site is included in the national GLP Compliance Programme and is inspected on a regular basis.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung/diesem Prüfstandort die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.

Based on the inspection report it can be confirmed, that this test facility / test-site is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Niedersächsisches Umweltministerium
Referat 33
Archivstraße 2
30169 Hannover

11. März 2002
Im Auftrage
Dr. Braedt

NIEDERSÄCHSISCHES UMWELTMINISTERIUM