AR226 - 1218

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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.

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

Alga, Growth Inhibition Test with *Scenedesmus subspicatus*, 72 h, Limit-Test with the Saturated Solution

acc. to OECD-Guideline No. 201 for Testing of Chemicals (June 1984)

Sponsor
Telomer Research Project
ASAHI GLASS
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CLARIANT
DAIKIN INDUSTRIES LTD
DUPONT

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Laboratory Project ID

Project-No.	020109CK
Study-No.	SSO84111

Study Completed On August 07, 2002

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- Alga, Growth Inhibition Test with *Scenedesmus subspicatus*, 72 h, Limit-Test with the Saturated Solution
Guideline	OECD-Guideline No. 201 for Testing of Chemicals (1984)
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)
Testing Facility	DR.U.NOACK-LABORATORIUM FÜR ANGEWANDTE BIOLOGIE Käthe-Paulus-Str.1, D-31157 Sarstedt Phone: (+49) 050 66/706 70, Fax: (+49) 05066/706 789, 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- Alga, Growth Inhibition Test with *Scenedesmus subspicatus*, 72 h, Limit-Test with the Saturated Solution
Guideline	OECD-Guideline No. 201 for Testing of Chemicals (1984)
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)
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	2002-04-07	2002-04-08
	2002-04-11	2002-04-11
	2002-04-12	2002-04-12
	2002-07-15	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 (Biologist)
Scientist:	Thomas Geffke (Chemical Engineer) Responsible for concentration control analysis
Technical Staff:	Ute Kutzner
Quality Assurance Unit:	Gudrun Möhrmann-Kalabokidis (Biologist)
Deputy:	Susanne Becker (Biologist)
Head of Testing Facility:	Dr. Udo Noack (Biologist)

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1 Summary

The acute toxicity of 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro- (batch number P00/001) to the unicellular freshwater green alga *Scenedesmus subspicatus* was determined according to the principles of OECD-Guideline 201, at DR.U.NOACK-LABORATORIUM FÜR ANGEWANDTE BIOLOGIE in 31157 Sarstedt, Germany from April 01 to 04, 2002.

The study was conducted under static conditions over a duration of 72 hours with an initial cell density of nominally 10^4 cells/mL and a saturated solution as limit concentration, containing 0.20 mg/L. 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.

Test vessels were coated with the test item for 14 days before the test started. Three replicates were tested for the limit concentration and 6 replicates for control. The test item was clearly dissolved throughout the test. Microscopic evaluation of the cells at the start and end of the incubation period revealed no morphological abnormalities. Water quality parameters of pH-value, measured at 0 and 72 h, and room temperature, measured continuously, were determined to be within the acceptable limits.

The concentration of the active ingredient 1-Decanol, 3,3,4,4,5,5,6,6, 7,7,8,8,9,9,10,10,10-heptadecafluoro- were analysed at the limit concentration level at test start and test end. Details of the analytical results are given in Part 10. 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 0.20 mg/L. At the end of the test the concentration of the test item was below the detection limit of 0.009 mg/L. Sorption to the glass walls of the test vessels is unlikely as the walls were already pre-coated for 14 days before the test has started.

The effects of 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro- based on initially measured a.i. concentration are summarized in Table 1.

Tab. 1: **NOEC/LOEC and EC_{50}-Values (0-72 h) of Biomass Inhibition and Rate-Related Inhibition**
NOEC/LOEC calculated using ANOVA (One Way Analysis of Variances) and DUNNETT'S test (α=0.05), based on initially measured concentration [mg/L] .

	Inhibition of biomass growth [mg/L]
E_bC_{50}	> 0.20
95 % confidence interval	Not applicable
LOEC	> 0.20
NOEC	0.20
	Rate-related inhibition [mg/L]
E_rC_{50}	> 0.20
95 % confidence interval	Not applicable
LOEC	> 0.20
NOEC	0.20

Conclusion

In this study 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro- was found not to inhibit the growth of the freshwater green alga *Scenedesmus subspicatus* at the saturated solution level, containing 0.20 mg/L (initially measured concentration).

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 / Active ingredient	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), 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) 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 No. 201 for Testing of Chemicals (adopted 7June, 1984)

TYPE AND PURPOSE OF THE STUDY

Alga, growth inhibition test for determination of the effects on growth rate and biomass production with a saturated solution of the test item over a period of 72 h (Limit Test).

TEST SYSTEM

Test organism	*Scenedesmus subspicatus* CHODAT SAG 86.81
Reason for the selection of the test system	According to the guideline *Scenedesmus subspicatus* CHODAT SAG 86.81 is suitable for this kind of study.
Origin	Sammlung von Algenkulturen (SAG), Pflanzenphysiologisches Institut der Universität Göttingen, Nikolausberger Weg 18, D-37073 Göttingen
Date of receipt	1997-04-07
Method of cultivation	Fresh stocks were prepared every month on Z-Agar. Light intensity amounted 35-70 $\mu E/m^2 \cdot s$ for 24 h per day.
Culture medium	Nutrient medium Z according to LÜTTGE et al. (1994).

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	The test item was heated to 50 °C before weighing for homogenisation.

Coating of the test vessels	A coating phase of 14 days was carried out.
Preparation	200 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 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 100 mL per test replicate was transferred from the clear liquid phase to 250 mL Erlenmeyer flasks and closed with plugs. Dilution water was used for the preparation of the coating solution.
Coating period	The readily filled and closed Erlenmeyer flasks were stored at nominally 23 ± 2 °C for 14 days. At the end of the coating period the flasks were emptied and used for the test.
Preparation of the test medium (saturated solution)	200 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 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. 100 mL per test replicate of the clear saturated solution was transferred to the coated Erlenmeyer flasks. The preparation method was chosen with regard to the test item properties and in accordance with the relevant guidance documents (OECD 2000, BAUA 2001).
CONTROL	Six replicates (without test item) were tested under the same test conditions as the test replicates.
SELF FLUORESCENCE	No self fluorescence was found at the saturated solution.
REFERENCE ITEM	Potassium dichromate p.a. (MERCK)
CAS No.	7778 - 50 - 9
Purity	100.2 %
Batch No.	K 233 78864646
Test concentrations (nom.)	0.1 - 0.2 - 0.4 - 0.8 - 1.6 - 3.2 mg/L

Range of validity	0.20 mg/L < E_bC_{50} (72 h) < 0.75 mg/L 0.60 mg/L < E_rC_{50} (72 h) < 1.03 mg/L
Experimental date	November 27 to 30, 2001

TEST CONDITIONS

Performance of the test	Static procedure
Exposure duration	Experimental start date: April 01, 2002 Experimental completion date: April 04, 2002
Replicates	Three replicates for limit concentration level, 6 per control
Test container	Erlenmeyerflasks, 250 mL, with cotton wool plugs
Test volume	100 mL
Dilution water	According to the guideline:

Component	Concentration [mg/L]
NH_4Cl	15
$MgCl_2 \cdot 6\ H_2O$	12
$CaCl_2 \cdot 2\ H_2O$	18
$MgSO_4 \cdot 7\ H_2O$	15
KH_2PO_4	1.6
$FeCl_3 \cdot 6\ H_2O$	0.08
$Na_2EDTA \cdot 2\ H_2O$	0.1
H_3BO_3	0.185
$MnCl_2 \cdot 4\ H_2O$	0.415
$ZnCl_2$	3×10^{-3}
$Na_2MoO_4 \cdot 2\ H_2O$	7×10^{-3}
$CoCl_2 \cdot 6\ H_2O$	1.5×10^{-3}
$CuCl_2 \cdot 2\ H_2O$	1×10^{-5}
$NaHCO_3$	50
pH	8.2 ± 0.2

Preculture

A four day old preculture was used as inoculum. Incubation was performed in 500 mL Erlenmeyer flasks with dilution water. For the start of the test the preculture was diluted with dilution water to receive an initial cell concentration of approximately 1×10^4 cells/mL in the replicates.
All algae were from the same source and have not been used in a previous study.

Application	At the test start fluorescence was measured after application of the test item.
Temperature	Nominally 23 ± 2 °C
Agitation	Test containers were placed on a rotary shaker and oscillated at approximately 70 rpm.
Light intensity	Nominally 60 - 120 $\mu E/m^2 \cdot s$
Light regime	24 h/d light

KIND AND FREQUENCY OF MEASUREMENT

Cell density was measured via Chlorophyll-a-fluorescence (excitation at 435 nm, emission at 685 nm). Each replicate was measured 6-fold. The cell density was measured at the beginning of the test and every 24 h. Filtrated dilution water was used as ground signal.

The pH-value at the beginning of the test was measured out of one additional replicate of the limit concentration and control. At the end it was measured from a pool of all replicates. The room temperature was measured continuously by a hygro-thermograph. Light intensities were measured prior to test start.

Microscopic evaluation of the cells was determined at the start and end of the incubation. Alga cells were checked for any unusual cell shapes, color differences, differences in chloroplast morphology, flocculations, adherence of algae to test vessels or aggregation of alga cells.

Equipment

pH-Meter, pH 191 (WTW)
Hygro-thermographe (KLIMATHERM)
Chlorophyll-impulsfluorometer (KLEINFELD)
Shaker, SM 25 A (BÜHLER)
Centrifuge: ZK 364, ZIRBUS
Inverse microscope (ZEISS IM 25)
Quantum Sensor (SKYE INSTRUMENTS LTD)
Rotating shaker 3040, GFL
Centrifuge: ZK 364, ZIRBUS
Fluorescent tubes, OSRAM L 36 W / 11-860, daylight (test)
Fluorescent tubes, LAMPI 18 W, 60 cm, warmwhite (culture)

DETERMINATION OF THE TEST ITEM

The test concentration level and control was analytically verified by gas chromatography with mass spectrometric detection (m/z = 463 and 395) at test start and test end. At the end of the test replicates with alga were analysed.
All details of the analytical method are given in part 10.

COURSE OF THE STUDY

Based on the results of a preliminary range finding test (NON-GLP), the definitive test was performed with 1 limit concentration level with 3 replicates.

A control with dilution water (without test item, six replicates) was tested under the same conditions as the test group.

A four day-old preculture incubated at study conditions was used for the main study.

Chlorophyll-fluorescence was determined at the beginning and after 24, 48 and 72 h.

Other measurements were performed as specified above.

CALCULATIONS AND STATISTICS

Cell densities for each replicate were calculated from the fluorescence values 3-6 (see raw data). The fluorescence values were related to cell density values as follows:

Calculation of cell density out of fluorescence values

$x = 63 \cdot Fq + 3625$

x = cell density cells/mL
63 = factor cells/mL/rel.units/10 mL
Fq = mean fluorescence value of all replicates per concentration rel.units/10 mL
3625 = cell density cells/mL
Correlation dated April 12, 2001, $r^2 = 0.9963$, n=8

The growth rate, the inhibition of biomass and the rate related inhibition after 72 h were calculated from the cell density values according to the formulas listed below.

Calculation of biomass integral

$$A = \frac{N_1 - N_0}{2} \cdot t_1 + \frac{N_1 + N_2 - 2 \cdot N_0}{2} \cdot (t_2 - t_1) + \ldots + \frac{N_{n-1} + N_n - 2\,N_0}{2} \cdot (t_n - t_{n-1}) \quad (1)$$

A = Area
N_0 = nominal number of cells/mL at time t_0
N_1 = measured number of cells/mL at time t_1
N_n = measured number of cells/mL at time t_n
t_1 = time of first measurement after beginning of test
t_n = time of n^{th} measurement after beginning of test

Calculation of biomass inhibition

$$I_A = ((A_C - A_T) / A_C) \cdot 100\ \% \quad (2)$$

I_A = biomass-inhibition %
A_C = biomass-integral in the control after n d in cells/mL
A_T = biomass-integral in the test concentration after n d in cells/mL

Calculation of growth rate

$$\mu = (\ln (N_n) - \ln (N_0)) / (t_n - t_0) \quad (3)$$

μ = growth rate cell density/d
N_n = biomass after t_n d in cells/mL
N_0 = biomass at t_0 in cells/mL
t_0 = time of beginning of test
t_n = time of n^{th} measurement after beginning of test

Calculation of rate related inhibition

$$I_{\mu t} = ((\mu_c - \mu_t) / \mu_c) \cdot 100\ \% \quad (4)$$

$I_{\mu t}$ = rate related inhibition %
μ_c = growth rate of the control after n d
μ_t = growth rate of the test concentrations after n d

NOEC, LOEC and statistical analysis

One way analysis of variance (ANOVA) , DUNNETT's and WILLIAM's test of growth rate and biomass integrals, respectively were carried out for the determination of statistically significant differences compared to control replicates. When running a one way analysis of variance a normality test and an equal variance test was done first. P-values for both normality and equal variance tests were 0.05. The α-value for ANOVA and DUNNETT's test (acceptable probability of incorrectly concluding that there is a difference) was α=0.05.

Software	The data for the tables in this report were computer generated and have been rounded for presentation from the full derived data. Consequently if calculated manually based on the given data minor variations may occur from these figures. Calculations were carried out using software - SigmaPlot rel. 2000 (2000), SPSS INC. - SigmaStat rel. 2.03 (1992-1997), SPSS INC. - Excel 2000 (2000), MICROSOFT CORPORATION - TOXSTAT rel. 3.4 (1994), Western Ecosystems Technology, Inc., Cheyenne, WY 82001 & David D. Gulley, University of Wyoming, WY, USA.
VALIDITY CRITERIA	The cell density had to increase at minimum 16-fold in the control replicates within 72 h. The temperature during the test had to be in the range of 21-25 °C controlled at ± 2 °C. The pH-value of the control replicates should not normally deviate by more than 1.0 units during the test.
DEVIATIONS FROM THE GUIDELINE	None
DEVIATIONS FROM THE STUDY PLAN	Purity was C8-perfluoroalkylethanol 99.3 % (a/a). The pH-value at the beginning of the test was measured out of one additional replicate. LOD and LOQ were 0.009 and 0.027 mg/L, respectively. These deviations had no impact on the quality and integrity of the study.
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 will be retained in a safe-deposit by Volksbank Sarstedt, D-31157 Sarstedt.

4 Results

4.1 Preliminary Test

The results of the preliminary test conducted February 25 to 28, 2002 are presented below:

Tab. 2: **Preliminary Test Results: Cell Density Values**
(2 replicates each, control: 4 replicates)

Dilution level	Replicate No.	Cell density [cells/mL] 0 h	72 h
1:1 of the saturated solution	1	10965	611103
	2	10697	471400
1:10 of the saturated solution	1	11705	537078
	2	11358	473763
Control	1	11626	586848
	2	11390	598818
	3	10587	626065
	4	11547	593463

No self fluorescence was found at the saturated solution

4.2 Definitive Test

Cell densities are listed in Table 4. Evaluations of biomass inhibition and rate-related inhibition are given in Table 5 (calculated out of cell density values). The growth inhibition effects of the test item are summarized in Table 3.

Microscopic evaluation of the cells at the start and end of the incubation period revealed no morphological abnormalities.

Water quality parameters of pH-value, measured at 0 and 72 h, and room temperature, measured continuously, met the guideline requirements.

Tab. 3: **NOEC/LOEC and EC_{50}-Values (0-72 h) of Biomass Inhibition and Rate-Related Inhibition**
NOEC/LOEC calculated using ANOVA (One Way Analysis of Variances) and DUNNETT'S test (α=0.05), based on initially measured concentration [mg/L] .

	Inhibition of biomass growth [mg/L]
E_bC_{50}	> 0.20
95 % confidence interval	Not applicable
LOEC	> 0.20
NOEC	0.20
	Rate-related inhibition [mg/L]
E_rC_{50}	> 0.20
95 % confidence interval	Not applicable
LOEC	> 0.20
NOEC	0.20

Tab. 4: **Cell Densities**
Based on Initially Measured Concentrations

Test item	Replicate No.	Cell Density [cells/mL]			
		0 h	24 h	48 h	72 h
0.20 mg/L	1	9075	39000	200563	609055
	2	9437	37724	227968	609685
	3	9453	39157	196594	587478
	mv	9322	38627	208375	602073
Control	1	9468	28242	165535	555505
	2	9342	31770	180624	598188
	3	9390	33219	177253	595038
	4	8602	32495	173190	631263
	5	9169	32904	189664	577398
	6	8854	32873	182010	637405
	mv	9138	31917	178046	599133

mv = mean value

Tab. 5: **Evaluation after 72 h**

Statistically significant differences of biomass integrals and growth rates compared to control values are marked (+), not significant differences are marked (-). NOEC was calculated using ANOVA (One Way Analysis of Variances), DUNNETT'S and WILLIAM's test (α=0.05), based on initially measured concentrations.

Test item	Replicate No.	Biomass Integral	Inhibition of Biomass [%]	Growth Rate	Rate-Related Inhibition [%]
0.20 mg/L	1	521403	- 7.13	1.40	- 0.56
	2	546942	- 12.38	1.39	0.35
	3	505858	- 3.94	1.38	1.28
	mv	524734	(+*) - 7.82	1.39	(-) 0.36
Control	1	447860		1.36	
	2	488133		1.39	
	3	484516		1.38	
	4	499812		1.43	
	5	488345		1.38	
	6	511451		1.43	
	mv	486685		1.39	

mv = mean value negative inhibitions = increase of growth

* significant difference caused by increase of growth

4.3 Test of the Reference Item

The acute toxicity of potassium dichromate to the unicellular freshwater green alga *Scenedesmus subspicatus* was determined over a period of 72 h from November 27 to 30, 2001.

Tab. 6: **EC_{50}-Values of Biomass Inhibition and Rate-Related Inhibition of the Reference Item**
based on nominal concentrations [mg/L], (0-72 h)

	Inhibition of biomass growth
E_bC_{50}	0.49
95 % confidence interval	0.45 - 0.54
	Rate-related inhibition
E_rC_{50}	0.94
95 % confidence interval	0.82 - 1.07

The EC_{50} - values of the reference item potassium dichromate after 72 h met the validity criteria according to EEC Directive 92/69/EEC Method C.3 Annex 2 (valid ranges are 0.20 – 0.75 mg/L and 0.60 – 1.03 mg/L potassium dichromate for inhibition of biomass growth and rate-related inhibition, respectively).

5 Validity Criteria

The study met the validity criteria of the guideline:

- The cell growth increased 66-fold after 72 h in the control (required: 16-fold).
- The temperature during the test was in the range of 21-25 °C.

  The difference from the mean was within ± 2 °C: min.: 22.0 °C, max.: 24.0 °C
  mean value: 23.0 °C.
- The pH-value in the control replicates increased not higher than 1.0 units: 8.13 to 8.54.

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- was not found to inhibit the growth of the freshwater green alga *Scenedesmus subspicatus* tested with a saturated solution, containing 0.20 mg/L (initially measured concentration).

7 Literature / References

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

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

(3) EEC Directive 92/69/EEC Method C.3, EEC L 383A/179 (December, 1992)

(4) LÜTTGE, U, SCHNEPF, E., LÄUCHLI, A., NAGATA, T. (editors, 1994): Botanica Acta, Journal of the German Botanical Society, No. 3 Volume 107 page 111-186 (June 1994), THIEME-VERLAG.

(5) OECD - guideline no. 201 for testing of chemicals: Alga, growth inhibition test. Adopted June 7, 1984

(6) OECD Series on Testing and Assessment, No 23 Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures ENV/JM/Mono (2000)6.

8 Graph

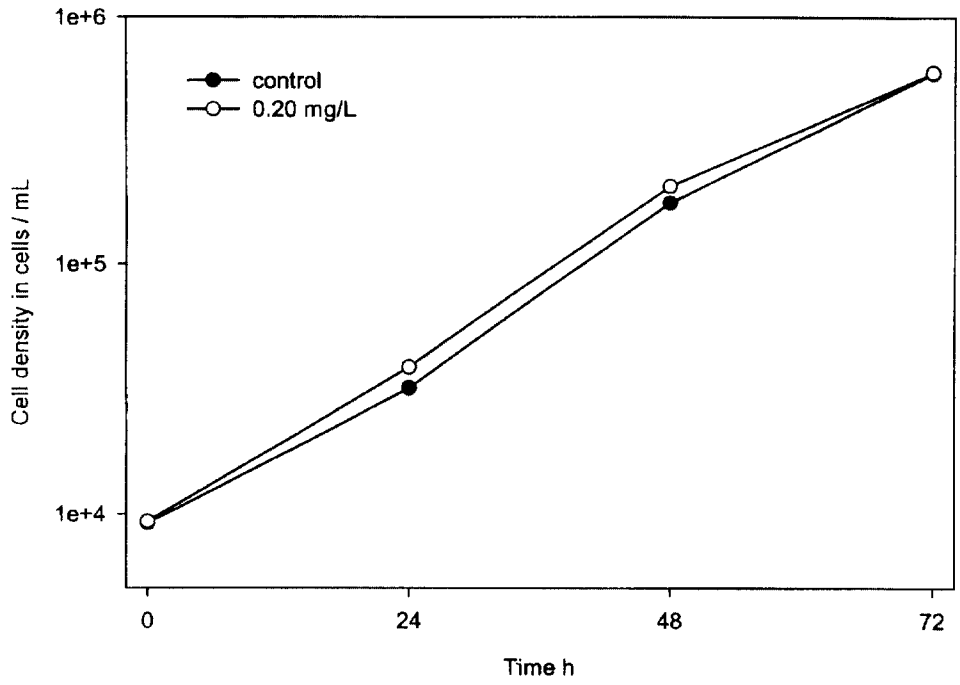


Fig. 1: **Cell Densities for Control and Limit Concentration (0-72 h)**

9 Physicochemical Data

Tab. 7: **Water Parameters**
Based on initially measured concentrations

	pH-value	
	Start; 0 h	End; 72 h
Control	8.13	8.54
0.20 mg/L	8.17	8.20
Temperature: min.: 22.0 °C max.: 24.0 °C mean value: 23.0 °C		

The mean light intensity was 67.2 µE/m^2 ·s (minimal 61.5, maximal 74.2 µE/m^2 ·s).

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

10.1 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 10.2.1.
PREPARATION OF SAMPLES	40 mL of the sample were extracted with 4.0 mL *tert.*-butyl methyl ether. 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:

$$T = W_{0.05} / (2 \cdot f)$$

T = Peak symmetry
$W_{0.05}$ = Peak width at 5 % of peak height [mm]
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.

10.2 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.

10.2.1 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 curve and chromatograms obtained for standard and test item are given in figures 2 - 7. For determination and detection limits see part 10.3.

10.2.2 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).

10.2.3 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]

	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)	
Serial No	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

10.2.4 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.

10.2.5 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

10.3 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		2002-04-01 Test Start	2002-04-04 Test End	
Start of Analysis		2002-04-05	2002-04-05	
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	0.17	< LOD	0.20
		0.18	< LOD	
	2	0.22	< LOD	
		0.22	< LOD	
Control		< LOD	< LOD	
		< LOD	< LOD	
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, because they were < LOD

10.4 Calibration Curves

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

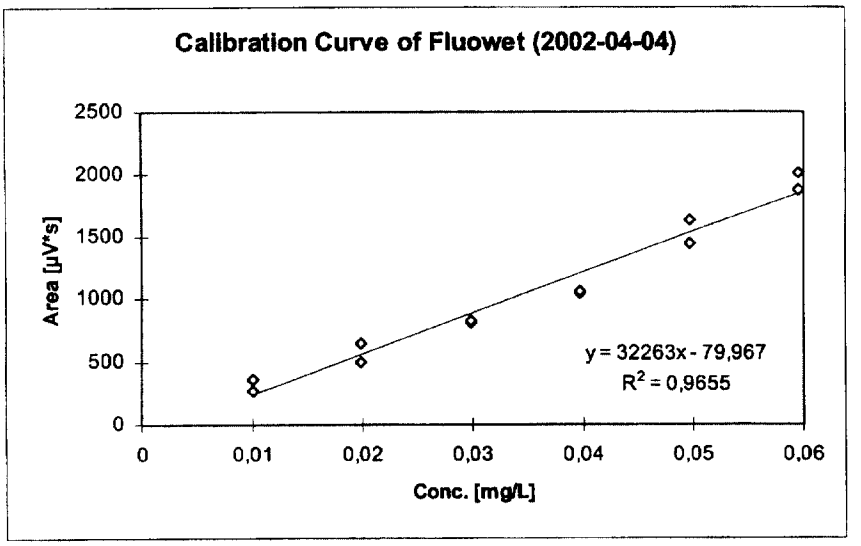


Fig. 2: **Calibration Curve of Standard - concentration range 0.01 - 0.06 mg/L** (dated 2002-04-04)

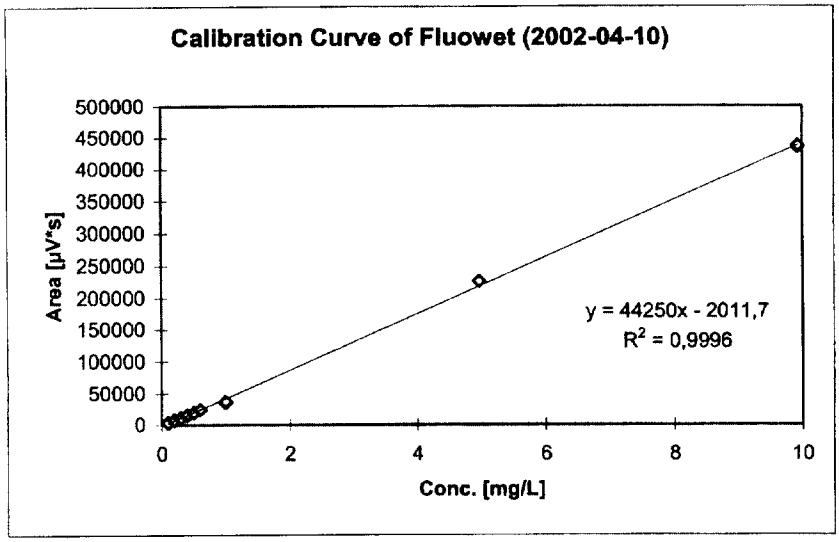


Fig. 3: **Calibration Curve of Standard - concentration range 0.1 - 10 mg/L** (dated 2002-04-10)

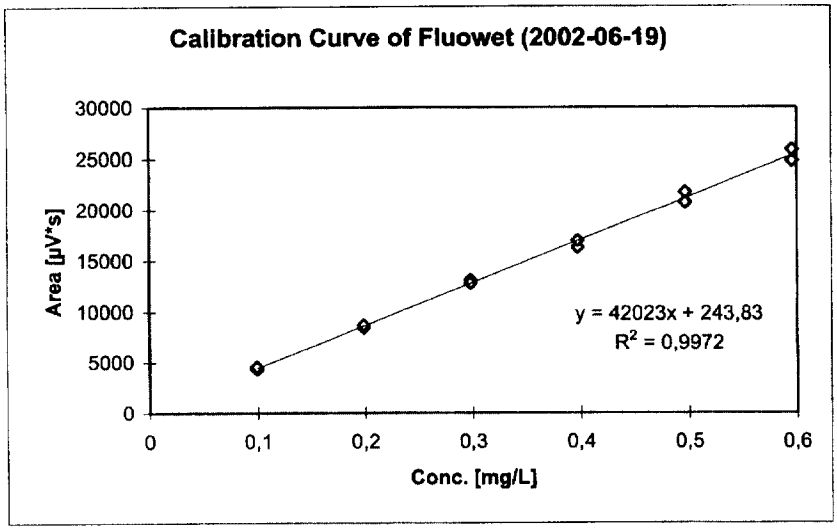


Fig. 4: **Calibration Curve of Standard - concentration range 0.1 - 0.6 mg/L** (dated 2002-06-19)

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.

10.5 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 l_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	4,916 +/- 0,200	4,911 min.	Pass
RT Offset		Δ0,00576 min.	
Peak Name		Fluowet	
CAS Number		678-39-7	
Status			
Quan Ions	69+895+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	

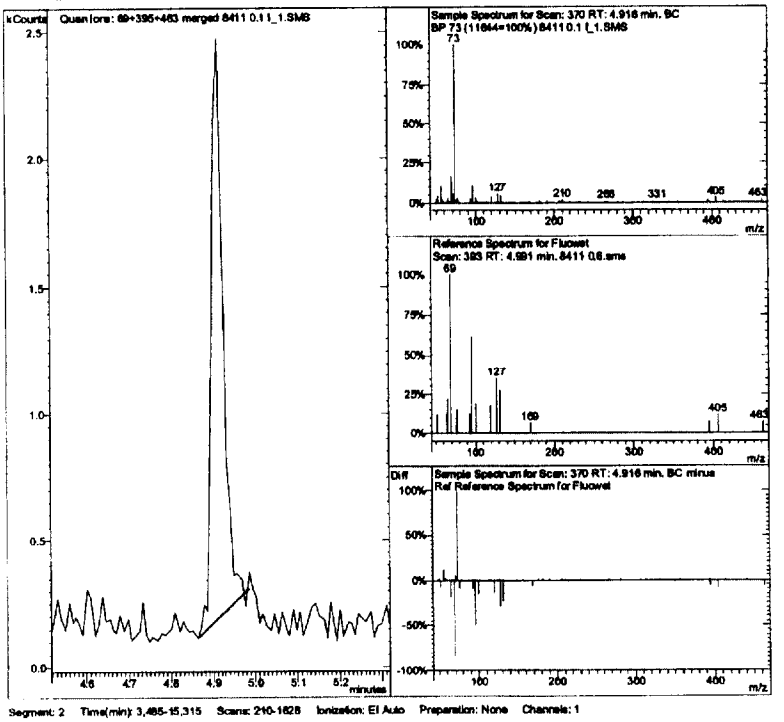


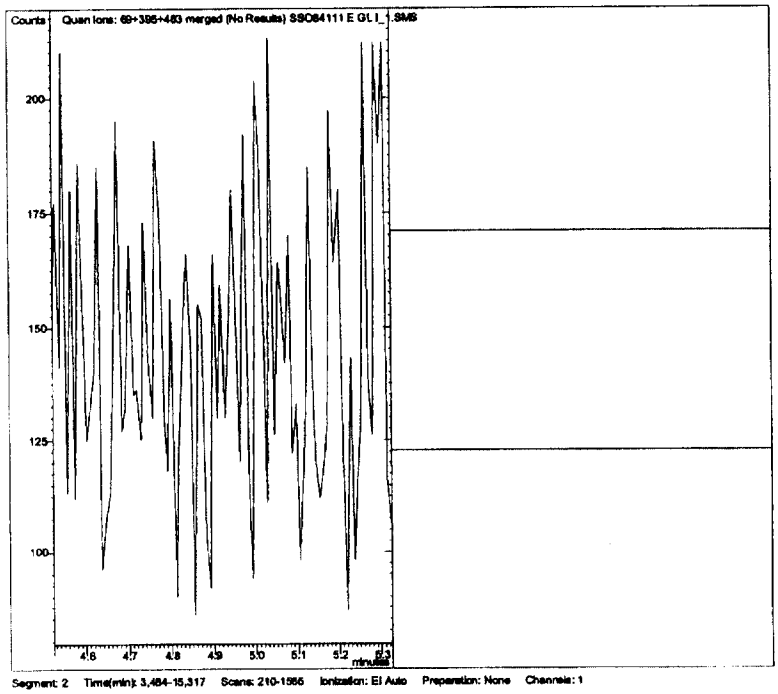
Fig. 5: **Chromatogram of Standard**
0.1 mg/L (dated 2002-04-10)

Target Compound Report for #1 from SSO84111 E GL I_1.SMS

Sample ID:	SSO84111 E GL I	Operator:	
Instrument ID:		Last Calibration:	05.04.02 13:46
Measurement Type:	Area	Calibration Type:	External Standard
Acquisition Date:	05.04.02 23:07	Data File:	c:\... sso84111 e gl i
Calculation Date:	08.04.02 08:39	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	4,925 +/- 0,200	5,082 min.	Pass
RT Offset		Δ0,15680 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	586	Fail
Calibration Equation	Linear, Ignore, None	y = +2,0285e+4x +630,0136	
Baseline Code			

Status and Errors: M : Missing peak.



Segment: 2 Time(min): 3,484-15,317 Scans: 210-1566 Ionization: EI Auto Preparation: None Channels: 1

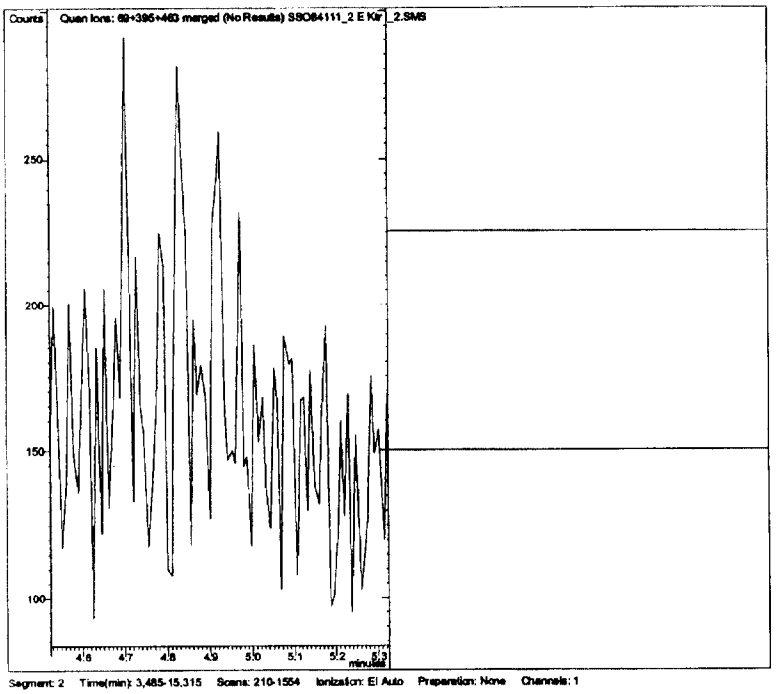
Fig. 6: **Chromatogram of Test Item**
Saturated solution at the test end (dated 2002-04-05)

Target Compound Report for #1 from SSO84111_2 E Ktr I_2.SMS

Sample ID:	SSO84111_2 E Ktr I	Operator:	
Instrument ID:		Last Calibration:	05.04.02 13:46
Measurement Type:	Area	Calibration Type:	External Standard
Acquisition Date:	05.04.02 22:45	Data File:	c:\... sso84111_2 e kt
Calculation Date:	08.04.02 08:39	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	4,925 +/- 0,200	4,926 min.	Pass
RT Offset		Δ0,00148 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	485	Fail
Calibration Equation	Linear, Ignore, None	y = +2,0285e+4x +630,0138	
Baseline Code			

Status and Errors: M: Missing peak.



Segment: 2 Time(min): 3,485-15,315 Scans: 210-1554 Ionization: EI Auto Preparation: None Channels: 1

Fig. 7: **Chromatogram of Control**
at test end (dated 2002-04-05)

11 Raw Data

Fluorescence at test beginning

Prüf-Nr. 020109CK

SB: Ku

Projekt-Nr. SSO84111

control

cuvette no.	F1	F2	F3	F4	F5	F6	mv F3-F6	corr. fluor.	VC [%]	overall mv
1	91	90	92	94	94	91	92,75	92,75	0,0182	87,5
2	88	87	87	92	94	90	90,75	90,75	0,0329	
3	92	85	88	90	97	91	91,5	91,5	0,0423	
4	76	73	78	83	77	78	79	79	0,0343	
5	85	85	87	90	88	87	88	88	0,0161	
6	80	82	83	82	82	85	83	83	0,0170	

conc.: **Saturated solution (0.21 mg/L)**

cuvette no.	F1	F2	F3	F4	F5	F6	mv F3-F6	corr. fluor.	VC [%]	overall mv
1	89	83	87	87	86	86	86,5	86,5	0,0067	90,41666667
2	89	90	92	91	96	90	92,25	92,25	0,0285	
3	89	88	90	94	93	93	92,5	92,5	0,0187	

Fluorescence after 24 h

Prüf-Nr. 020109CK

SB: Ku

Projekt-Nr. SSO84111

control

cuvette no.	F1	F2	F3	F4	F5	F6	mv F3-F6	corr. fluor.	VC [%]	overall mv
1	387	375	388	381	389	405	390,75	390,75	0,0260	449,0833333
2	459	442	457	441	436	453	446,75	446,75	0,0221	
3	481	471	475	474	468	462	469,75	469,75	0,0128	
4	460	470	467	448	462	456	458,25	458,25	0,0179	
5	451	471	461	464	460	474	464,75	464,75	0,0138	
6	474	472	465	455	465	472	464,25	464,25	0,0151	

conc.: **Saturated solution (0.21 mg/L)**

cuvette no.	F1	F2	F3	F4	F5	F6	mv F3-F6	corr. fluor.	VC [%]	overall mv
1	571	582	566	563	564	553	561,5	561,5	0,0103	555,5833333
2	532	528	537	547	544	537	541,25	541,25	0,0093	
3	590	575	561	561	564	570	564	564	0,0075	

Fluorescence after 48 h

Prüf-Nr. 020109CK

SB: Ku

Projekt-Nr. SSO84111

control

cuvette no.	F1	F2	F3	F4	F5	F6	mv F3-F6	corr. fluor.	VC [%]	overall mv
1	2668	2598	2602	2592	2546	2540	2570	2570	0,0123	2768,583333
2	2782	2834	2822	2826	2804	2786	2809,5	2809,5	0,0065	
3	2672	2788	2768	2768	2756	2732	2756	2756	0,0082	
4	2676	2720	2732	2684	2686	2664	2691,5	2691,5	0,0107	
5	2942	2996	2986	2940	2924	2962	2953	2953	0,0091	
6	2856	2824	2836	2812	2854	2824	2831,5	2831,5	0,0063	

conc.: **Saturated solution (0.21 mg/L)**

cuvette no.	F1	F2	F3	F4	F5	F6	mv F3-F6	corr. fluor.	VC [%]	overall mv
1	3180	3086	3182	3116	3102	3104	3126	3126	0,0121	3250
2	3478	3520	3592	3510	3600	3542	3561	3561	0,0120	
3	3008	3110	3062	3046	3068	3076	3063	3063	0,0041	

Fluorescence after 72 h

Prüf-Nr. 020109CK

SB: Ku

Projekt-Nr. SSO84111

control

cuvette no.	F1	F2	F3	F4	F5	F6	mv F3-F6	corr. fluor.	VC [%]	overall mv
1	9140	9040	8820	8900	8630	8690	8760	8760	0,0140	9452,5
2	9770	9720	9610	9430	9410	9300	9437,5	9437,5	0,0136	
3	9690	9760	9450	9510	9330	9260	9387,5	9387,5	0,0121	
4	9800	10030	10170	10090	9940	9650	9962,5	9962,5	0,0230	
5	9060	9120	9250	9170	9060	8950	9107,5	9107,5	0,0144	
6	9730	10120	10140	10080	10170	9850	10060	10060	0,0144	

conc.: **Saturated solution (0.21 mg/L)**

cuvette no.	F1	F2	F3	F4	F5	F6	mv F3-F6	corr. fluor.	VC [%]	overall mv
1	9810	10080	9800	9650	9570	9420	9610	9610	0,0165	9499,166667
2	9670	9850	9730	9560	9460	9730	9620	9620	0,0139	
3	9220	9420	9430	9310	9250	9080	9267,5	9267,5	0,0157	

12 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 Perfluoralkylethanol 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

13 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