Final Report

Original 2 of 2

Determination of the Inhibition of the
Respiration of Activated Sludge when exposed to
3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctyl acrylate
according to OECD 209 resp. EU C.11

Study No.: 12100104G701

Sponsor:
Daikin Industries, Ltd.
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Monitor:

Test Facility:
LAUS GmbH
Auf der Schafweide 20
D-67489 Kirrweiler
Germany

Study Director:

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1 GLP-Compliance Statement

It is hereby declared that all tests were made in accordance with the „Revised OECD Principles of Good Laboratory Practice" (Paris, 1997) as stated in the following guidelines:

- OECD Principles of Good Laboratory Practice, adopted by Council on 26th November 1997; Environment Directorate, Organisation for Economic Cooperation and Development, Paris 1998
- Directive 2004/10/EC of the European Parliament and of the Council of 11 February 2004 on the harmonisation of laws, regulations and administrative provisions relating to the application of the principles of good laboratory practice and the verification of their applications for tests on chemical substances (codified version)
- Chemikaliengesetz (Chemicals Act) of the Federal Republic of Germany (ChemG) § 19(a) to §19(d) and annexes 1 and 2, version 02 July 2008, Federal Law Gazette, Germany (BGBL) N. 28/2008, pp. 1146-1184, last amended in Federal Law Gazette, Germany (BGBL) from 24. Feb. 2012, N. 10/2012, pp. 212, 263

Responsibility for the accuracy of the information concerning the test item as well as for its authenticity rests with the sponsor.

I herewith accept responsibility for the data presented within this report.

There were no circumstances that may have affected the quality or integrity of the study.

This report contains the following data which was not acquired under GLP conditions:
Analysis of drinking water, delivered by Verbandsgemeinde Maikammer

18 DEC 2012

Date

Study Director

Information on Study Organisation:

Study Plan dated	29. Nov. 2012
Experimental Starting Date	04. Dec. 2012
Experimental Completion Date	05. Dec. 2012
Draft Report dated	10. Dec. 2012

2 Quality Assurance Unit Statement

This study has been inspected by the quality assurance unit according to the principles of Good Laboratory Practice. Study Plan and Final Report were checked at the dates given below, the Study Director and the management were informed with the corresponding report.

Also, the performance of the study was inspected, and findings were reported to Study Director and management. The inspection of short-term studies (duration less than four weeks) is carried out as audit of process concerning major technical phases of at least one similar test. Frequency is once or more a quarter.

The study was conducted and the reports were written in accordance with the Study Plan and the Standard Operating Procedures of the test facility.

Deviations from the Study Plan were acknowledged and assessed by the Study Director and included in the Final Report.

The reported results reflect the raw data of the study.

Verified Procedure	Inspected on	Findings reported on	Audit report no.
Study plan	21. Nov. 2012	21. Nov. 2012	121121-06
Performance of study (audits of process, studies 12081001G701, 12072302G701, 12092001G701)	05. Oct. 2012 30. Oct. 2012 13. Nov. 2012	05. Oct. 2012 30. Oct. 2012 13. Nov. 2012	121005-11 121030-07 121113-06
Draft report	13. Dec. 2012	13. Dec. 2012	121213-08
Final report	18. Dec. 2012	18. Dec. 2012	121218-10

18 DEC 2012

Date

Quality Assurance Manager

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3 SUMMARY

Title of Study: Determination of the Inhibition of the Respiration of Activated Sludge when exposed to 3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctyl acrylate according to OECD 209 resp. EU C.11

Findings and Results:

One valid experiment was performed.

Because volatility of the test item could not be excluded, the experiment was performed in closed Schott flasks without aeration and with analytical determination of the content of test item in the liquid phase at the beginning and at the end of the test, as described in the OECD guideline.

The study was performed using five concentrations, ranging from 1000 to 10 mg/L nominal concentration. The dry matter of the activated sludge was determined as 2.04 g suspended solids/L, giving a concentration of 1.02 g suspended solids/L in the test. EC50 of the positive control was determined as 7.8 mg/L (95% confidence interval: 5.2 – 50 mg/L), which lies within the demanded range of 2 – 25 mg/L.

Because of slow dissolution of test item, the measured concentrations at the end of the test were much higher than at the beginning.

At no test item concentration, any inhibition effect could be observed.

The following results for the test item 3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctyl acrylate were determined:

3h NOEC ≥ 1000 mg/L nominal concentration
3h EC10 > 1000 mg/L nominal concentration
3h EC50 > 1000mg/L nominal concentration

4 PURPOSE AND PRINCIPLE OF THE STUDY

This study was performed in order to evaluate the effect of 3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctyl acrylate on the respiration of activated sludge obtained from a domestic sewage treatment plant. The Activated Sludge Respiration test is a rapid screening method to identify substances which may adversely affect aerobic micro-organisms.

The effects on micro-organisms from activated sludge were determined by measuring their respiration rate under defined conditions in the presence of different concentrations of the test substance. The respiration rates of samples of activated sludge fed with synthetic sewage were measured in an enclosed cell containing an oxygen electrode after a contact time of three hours. The sensitivity of each batch of activated sludge was checked with a positive control.

Sponsor's intent: registration in accordance with: REACH.

5 LITERATURE

The study was conducted in accordance with the following guidelines:

- OECD Guidelines for the Testing of Chemicals No. 209 adopted 22. Jul. 2010 "Activated Sludge, Respiration Inhibition Test"
- EU-Method C.11, "Biodegradation - Activated Sludge Respiration Inhibition Test", adopted 30 May 2008

Corresponding SOP of LAUS GmbH

- SOP 118 007 01 edition 6, valid from 01. Jun. 2011 and edition 7, valid from 03. Dec. 2012: "Belebtschlamm-Atemhemmtest"

6 MATERIALS AND METHODS

6.1 Test Item

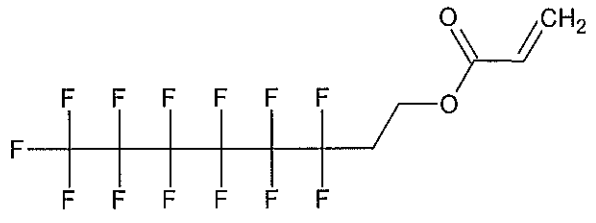
Designation in Test Facility:	12100104G
Date of Receipt:	01. Oct. 2012
Condition at Receipt:	room temp., in proper conditions

6.1.1 Specification

The following information concerning identity and composition of the test item was provided by the sponsor.

Name	3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctyl acrylate
Batch no.	6SFCC96119
Appearance	colourless liquid
Composition	3,3,4,4,5,5,6,6,7,7,8,8,8- Tridecafluorooctyl acrylate
CAS No.	17527-29-6
EINECS-No.	241-527-8
Molecular formula	not stated
Molecular weight	not stated
Purity	99.5%(GC)
Homogeneity	not stated
Vapour pressure	not stated
Stability	not stated
Solubility	not stated
Production date	Jun. 2009
Expiry date	24. Sep.2013
Storage	Room Temperature: (20 ± 5°C)
Hazard information	Xi irritant
R-phrases	R36/37/38: Irritating to eyes, respiratory system and skin.
S-phrases	S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S37: Wear suitable gloves.

6.1.2 Structure



C=CC(=O)OCCC(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F

6.1.3 Storage

The test item was stored in a closed vessel dry at room temperature.

6.1.4 Preparation of Test Solution

None, the test item was added to the test vessels directly.

6.2 Positive Control

3.5-Dichlorophenol (CAS 591-35-5) was used as positive control. A stock solution in deionised water containing 500 mg/L (nominal) was freshly prepared for the experiment.

6.3 Test System

Activated sludge from a biologic sewage treatment plant was used. The chosen plant treats mostly domestic sewage.

6.3.1 Source and Pre-Treatment

The sludge was taken from the activation basin of the ESN (Stadtentsorgung Neustadt) sewage treatment plant D-67435 NW-Lachen-Speyerdorf.

Upon arrival in the test facility, the sludge was filtrated, washed with tap water and re-suspended in tap water. The activated sludge was aerated until usage in the test and fed daily with 50 mL /L synthetic sewage feed.

6.3.2 Specification

The specification of the test system is given in the following table.

Table 6.3-a Sludge Characterisation

Date of Collection	Date of Experiment	pH	Dry matter of Sludge (g suspended solids/litre)	Dry matter in the Test (g suspended solids/litre)
03. Dec. 2012	04. Dec. 2012	7.6	2.04	1.02

6.4 Chemicals and Nutrient Solution

All chemicals used in the test were "analytical grade" or "for use in microbiology".

Composition of nutrient solution (synthetic sewage):

Peptone	16.0 g
Meat Extract	11.0 g
Urea	3.0 g
NaCl	0.7 g
$CaCl_2*2H_2O$	0.4 g
$MgSO_4*7H_2O$	0.2 g
K_2HPO_4	2.8 g
Deionised water	ad 1000 mL

The pH of the solutions were 7.0 and 7.1 and therefore within the range of 7.5 ±0.5.

The nutrient solution was frozen immediately after preparation.

6.5 Dilution Water

Tap water (composition see Annex 2, page 25).

6.6 Test Vessels and Measuring Flasks

2000 ml Schott flasks, closed with Teflon seals and lids were used as test vessels. They were cleaned before use following SOP 114 003 01 in the current edition.

Narrow-neck glass bottles with flat bottoms were used as measuring flasks.

6.7 Instruments and Devices

The following instruments and devices were used in the performance of the study:

- Analytical scales Mettler Toledo XS205 DU LAUS No. 2
- Precision scales Mettler XS 6001S
- pH-Meter 3310 wtw
- Adjustable pipettes with one-way tips, LAUS No. 29
- Oxygen meter Oxi 538 with sensor CellOX 325
- Magnetic stirrer
- Drying Chamber Memmert LAUS No. 4
- PC LAUS No. WKS 025
- Gas chromatograph GC 3 hp 6890N with flame ionisation detector (FID)

Volumetric measurement material (glass) and standard laboratory equipment was also used. Usage and, if applicable, calibration of all instruments following the corresponding SOP in the current edition.

6.8 Analytical Method

The content of the test item 3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctyl acrylate in the test solutions was determined with a validated GC/FID Method.

The method is fully described in validation report VB12100104G926. This document and the corresponding raw data will be archived following GLP regulations.

6.8.1 Method Characterisation

Analytical instrument	GC hp 6890N LAUS No. 3 with FID
Retention time:	3.94 - 3.95 min
Injection volume:	1 µL
Calibrated range:	1 – 80 mg/L
Process variation coefficient	2.42 %
Recovery from test medium	62 % (nominal concentration 100 mg/L)
	51 % (nominal concentration 10 mg/L)
Stability in test medium	65 % after 0.5 h (1000 mg/L)
	72 % after 3 h (1000 mg/L)
	2 % after 0.5 h (10 mg/L)
	0 % after 3 h (10 mg/L)

For determination of recovery from medium and stability in medium, the test medium was spiked with a stock solution containing 1000 mg/L test item in methanol. Stability of the test item under test conditions (open glass beakers with aeration) was tested using two different concentrations, 1000 mg/L (far above the limit of solubility in activated sludge respiration inhibition test medium) and 10 mg/L.

At 1000 mg/L, the test item showed sufficient stability after storage for 0.5 and 3 hours.

At 10 mg/L, only 2 % of the test item was found after 0.5 hours.

The apparent stability at 1000 mg/L might be caused by additional supply of previously undissolved test item. It might be possible that loss of test item is caused by volatilization. Therefore, the test was performed in a closed system without aeration.

6.8.2 Instrumental Parameters

Column	Rtx-440, 30 m * 0.25 mm * 0.25 µm
Temperature	50 °C/1 min. isothermal, 20 °C/min. to 300 °C
Gas Type	H_2
Inlet	280 °C, splitless
Detector	FID, 300 °C

6.8.3 Calibration Curve

For the calibration, a dilution series in methyl t-butyl ether was prepared, using a stock solution.

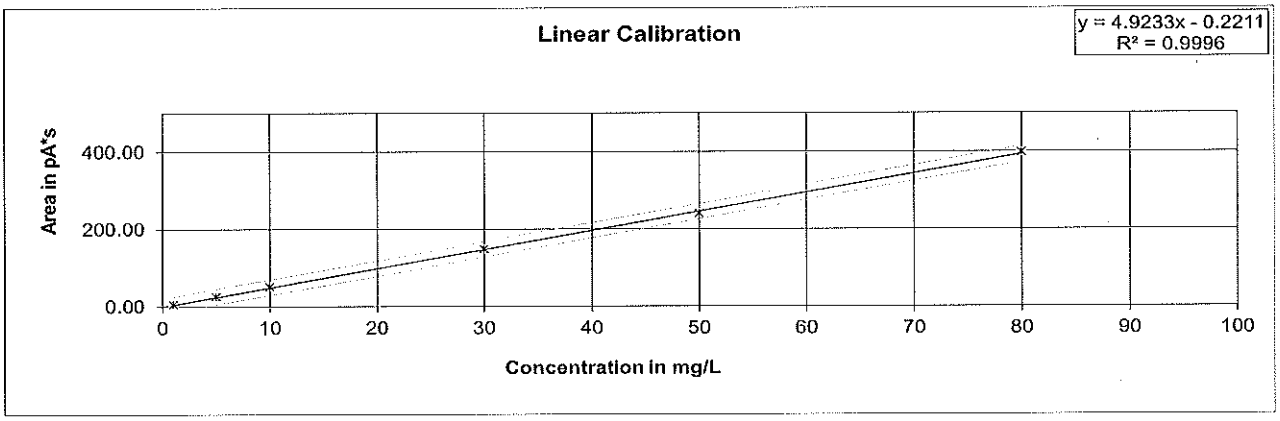


Table 6.8-a Parameters of Calibration

Parameter	Value	Unit
Calibration Range	1 – 80	mg/L
Slope	4.92327079	pA*s / mg/L
Intersection y-axis	-0.22106209	pA*s
Residual standard deviation	3.492101021	pA*s
Method standard deviation	0.709305088	mg/L
Method variation coefficient	2.42	%
Correlation coefficient r	0.999788823	

For each measurement, the settings and parameters were documented.

All data is archived following GLP regulations.

6.8.4 Sample Preparation

6.8.4.1 Concentrations 1000 mg/L - 32 mg/L, nominal
10 mL of the respective solution in medium was added to 90 mL demineralised water with 5 g NaCl; then, the solution was extracted two times with the solvent methyl t-butyl ether (9 mL, 4 mL), the organic phase was collected after drying with Na_2SO_4 into a 10 mL flask and the flask was filled to 10 mL with methyl t-butyl ether.

6.8.4.2 Concentration 10 mg/L, nominal
30 mL of the solution in medium was added to 70 mL demineralised water with 5 g NaCl; then, the solution was extracted two times with the solvent methyl t-butyl ether (9 mL, 4 mL), the organic phase was collected after drying with Na_2SO_4 into a 10 mL flask and the flask was filled to 10 mL with methyl t-butyl ether. Threefold enrichment was achieved.

7 CONDUCT OF THE STUDY, FINDINGS AND RESULTS

7.1 Preparations

On the day before the experiment, the inoculum was taken from its source, washed, aerated and the dry matter was determined. Volume was adapted to the desired content of dry matter. The nutrient solution was thawed and the sludge was fed with 50 mL/L sludge.

On the day of the experiment, the dry matter was determined once more. The stock solution of the positive control was prepared.

7.2 Experimental Parameters

Duration	three hours
Replicates	one replicate/treatment (positive control), five replicates/treatment (test item)
Control	two replicates before and two after measuring positive control and test item, respectively
Water	tap water
Aeration	purified air, using Pasteur pipettes, flow approx. 0.75 L/min.
Feeding	nutrient solution, 16 mL/vessel
Temperature	20.5 – 21.2 °C

7.3 Description of Performance

The study was performed in a closed system without aeration but with analytical determination. In the control vessels, 16 mL nutrient solution was mixed with 234 mL water. The positive control and the test vessels were prepared by putting the appropriate amount of positive control solution respectively test item into the test vessel, adding 16 mL nutrient solution and water to give 250 mL. Then, 250 mL inoculum was added in five minute intervals. After addition of the inoculum, the flasks were closed immediately and the mixtures were stirred without aeration.

After three hours, the content of the first vessel was poured in a 250 mL narrow-neck bottle and the respiration rate was determined by measurement of the O_2-concentration over a period of max. five minutes[1]. The following vessels were measured likewise in five minute intervals.

At the beginning and at the end samples for analytical determination were taken. For this, a 50 mL glass flask was completely filled with test solution and immediate closed.

[1] The respiration rate was measured over a period of only five minutes. In the guideline, a period of "up to ten minutes" is recommended. As the linearity of all regression linear curves (r^2 of 0.99 or more) is given, the measurement period can be considered as sufficiently long.

7.4 Calculations

7.4.1 O_2-Consumption

The oxygen consumption rate is calculated from the slope of the linear part of the oxygen consumption curve (approx. between 6.5 and 2.5 mg O_2/L) using linear regression as stated in the following equation:

$$R = \frac{\left[\sum_{i=1}^{i=n} \frac{\rho_{i-1} - \rho_i}{\Delta t} * 60\right]}{n}$$

with:

ρ_{i-1} value of first measurement of dissolved oxygen in mg/L
ρ_i value of second measurement of dissolved oxygen in mg/L
Δt time interval between both measurements in minutes
n number of oxygen measurements of the respective replicate

7.4.2 Calculation of Inhibition

The percentage inhibition was calculated from:

$$I = (1 - \frac{R_T}{R_C}) * 100$$

with:

R_T oxygen consumption rate of the treatment
R_C mean oxygen consumption rate of controls

7.4.3 Calculation of EC50

The estimation of the EC50 was accomplished using the software Origin™. The calculated values for r resp. r^2 are given in the graphs.

The data were evaluated using linear fit on a probability-logarithmic scale.

Equation: Prob (y) = A + B * log (x)

with

y inhibition in %
x concentration in mg/L

7.5 Data

The experiment was performed on 04. Dec. 2012.

7.5.1 Content of Test Vessels

Concentration of the positive control was calculated using the concentration of the stock solution and the dilution factor.

The test item was added to the test vessels directly.

The content of the test vessels is given in the following table.

Table 7.5-a Content of Test Vessels (continued on next page)

Number	Content	Nom. Concentration (mg/L)	Nutrient Solution (mL)	Tap Water (mL)	Stock Solution added (mL)	Concentration of Stock Solution (mg/L)	Inoculum (mL)
1-2	*	*	*	*	*	*	*
3	Control	0	16	234	0	--	250
4	Control	0	16	234	0	--	250
5	Positive Control	5	16	229	5	507	250
6	Positive Control	10	16	224	10	507	250
7	Positive Control	20	16	214	20	507	250
8	Positive Control	40	16	194	40	507	250
9	Control	0	16	234	0	--	250
10	Control	0	16	234	0	--	250
Number	**Content**	**Nom. Concentration (mg/L)**	**Nutrient Solution (mL)**	**Tap Water (mL)**	**Amount added (mg)**		**Inoculum (mL)**
11	Test Item	1000.2	16	234	500.1	--	250
12	Test Item	1002.2	16	234	501.1	--	250
13	Test Item	1002.0	16	234	501.0	--	250
14	Test Item	1000.4	16	234	500.2	--	250
15	Test Item	1000.6	16	234	500.3	--	250
16	Test Item	320.8	16	234	160.4	--	250
17	Test Item	321.0	16	234	160.5	--	250
18	Test Item	320.2	16	234	160.1	--	250
19	Test Item	321.6	16	234	160.8	--	250
20	Test Item	321.6	16	234	160.8	--	250
21	Test Item	101.8	16	234	50.9	--	250
22	Test Item	101.4	16	234	50.7	--	250
23	Test Item	100.8	16	234	50.4	--	250
24	Test Item	100.0	16	234	50.0	--	250
25	Test Item	100.8	16	234	50.4	--	250

*** other test item was measured in vessels 1 – 2**

Table 7.5-b Content of Test Vessels (continued from previous page)

Number	Content	Nom. Concentration (mg/L)	Nutrient Solution (mL)	Tap Water (mL)	Amount added (mg)	Inoculum (mL)
26	Test Item	33.6	16	234	16.8	250
27	Test Item	32.0	16	234	16.0	250
28	Test Item	32.2	16	234	16.1	250
29	Test Item	32.8	16	234	16.4	250
30	Test Item	33.0	16	234	16.5	250
31	Test Item	11.0	16	234	5.5	250
32	Test Item	11.2	16	234	5.6	250
33	Test Item	10.0	16	234	5.0	250
34	Test Item	11.6	16	234	5.8	250
35	Test Item	11.0	16	234	5.5	250
36	Control	0	16	234	0	250
37	Control	0	16	234	0	250

7.5.2 Time Schedule

The time schedule is presented in the following table.

Table 7.5-c Time Schedule

No.	Start of Test	Begin of O_2-Measurement	End of O_2-Measurement
1-2	*	*	*
3	10:40	13:40:38	13:45:03
4	10:45	13:45:28	13:50:03
5	10:50	13:50:25	13:55:13
6	10:55	13:55:38	14:00:04
7	11:00	14:00:44	14:05:04
8	11:05	14:05:34	14:10:01
9	11:10	14:10:21	14:15:09
10	11:15	14:15:26	14:19:56
11	11:20	14:20:32	14:25:02
12	11:25	14:25:27	14:29:52
13	11:30	14:30:22	14:34:57
14	11:35	14:35:32	14:40:02
15	11:40	14:40:32	14:44:57
16	11:45	14:45:27	14:49:57
17	11:50	14:50:32	14:55:02
18	11:55	14:55:27	15:00:09
19	12:00	15:00:27	15:05:02
20	12:05	15:05:32	15:10:02
21	12:10	15:10:22	15:14:52
22	12:15	15:15:39	15:20:02
23	12:20	15:20:27	15:24:49
24	12:25	15:25:14	15:29:57
25	12:30	15:30:19	15:34:49
26	12:35	15:35:14	15:40:02
27	12:40	15:40:19	15:44:49
28	12:45	15:45:27	15:50:02
29	12:50	15:50:32	15:55:14
30	12:55	15:55:27	16:00:01
31	13:00	16:00:31	16:05:01
32	13:05	16:05:26	16:10:14
33	13:10	16:10:39	16:14:51
34	13:15	16:15:21	16:19:56
35	13:20	16:20:21	16:24:56
36	13:25	16:25:21	16:30:04
37	13:30	16:30:26	16:35:16

*** other test item was measured in vessels 1 – 2**

7.5.3 O_2-Consumption, Inhibition

The values of the O_2 consumption (which is a measure for the viability of the bacteria) of controls, test and positive control and the calculated inhibition are presented in the following table. The measured pH at the end of the test is also stated.

Table 7.5-d O2-Consumption, Inhibition

Vessel No.	Content	Concentration in mg/L	O_2 consumption in mg/(L*min.)	O_2 consumption in mg/(L*h)	Inhibition in %	pH
1-2	*	*	*	*	*	*
3	Control	0	0.4753	28.519	0	7.8
4	Control	0	0.4765	28.587	0	7.8
5	Positive Control	5	0.3462	20.774	31.2	7.7
6	Positive Control	10	0.1926	11.556	61.7	7.6
7	Positive Control	20	0.0790	4.739	84.3	7.6
8	Positive Control	40	0.0338	2.027	93.3	7.7
9	Control	0	0.5309	31.854	0	7.8
10	Control	0	0.4964	29.784	0	7.8
11	Test Item	1000.2	0.5298	31.787	-5.3	7.7
12	Test Item	1002.2	0.5488	32.926	-9.1	7.7
13	Test Item	1002.0	0.5656	33.938	-12.4	7.7
14	Test Item	1000.4	0.5540	33.242	-10.1	7.8
15	Test Item	1000.6	0.5899	35.395	-17.2	7.8
16	Test Item	320.8	0.5588	33.530	-11.1	7.7
17	Test Item	321.0	0.5337	32.025	-6.1	7.7
18	Test Item	320.2	0.5888	35.328	-17.0	7.7
19	Test Item	321.6	0.5378	32.269	-6.9	7.7
20	Test Item	321.6	0.5512	33.070	-9.5	7.8
21	Test Item	101.8	0.5442	32.650	-8.1	7.7
22	Test Item	101.4	0.5208	31.250	-3.5	7.8
23	Test Item	100.8	0.4640	27.839	7.8	7.7
24	Test Item	100.0	0.5249	31.495	-4.3	7.7
25	Test Item	100.8	0.4824	28.942	4.1	7.7
26	Test Item	33.6	0.5616	33.693	-11.6	7.7
27	Test Item	32.0	0.5651	33.905	-12.3	7.7
28	Test Item	32.2	0.5394	32.366	-7.2	7.8
29	Test Item	32.8	0.5391	32.348	-7.1	7.7
30	Test Item	33.0	0.5603	33.617	-11.3	7.7
31	Test Item	11.0	0.5966	35.795	-18.6	7.8
32	Test Item	11.2	0.5627	33.763	-11.8	7.7
33	Test Item	10.0	0.5596	33.576	-11.2	7.7
34	Test Item	11.6	0.5580	33.480	-10.9	7.7
35	Test Item	11.0	0.4967	29.802	1.3	7.8
36	Control	0	0.5021	30.127	0	7.8
37	Control	0	0.5380	32.282	0	7.8

*** other test item was measured in vessels 1 – 2**

7.5.4 Analytical Determinations

The test item concentration was analysed in one replicate per treatment at the beginning and at the end of the test. Because of slow dissolution of the test item, only marginal test item concentrations were measured at the beginning. Surprisingly, in the highest concentrated treatment, no test item could be detected at the beginning of the experiment. This might be caused by insufficient homogenisation of the sample. After three hours stirring, significantly higher test item concentrations could be measured in all treatments.

During validation of the analytical method, the solutions for determination of recovery and stability in medium were prepared using methanol as solvent. In the main study, no solvent was used because usage of solvents is not described in the guideline. Therefore, due to the slow dissolution, the actual test item concentration during the test is not known. Therefore, the nominal concentrations were used for determination of the results.

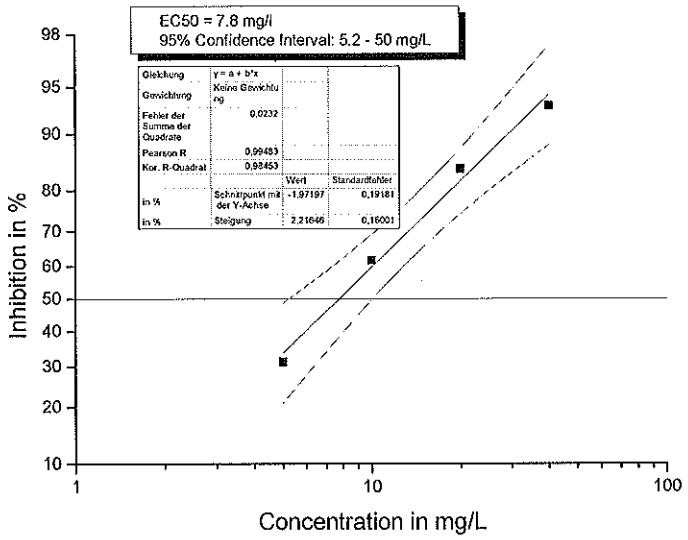
The measured concentrations and the corresponding recovery rates are given in the following table:

Table 7.5-e Measured Concentrations

Nominal Conc. in mg/L	Measured Conc. in mg/L	
	0 h	3 h
10	1.5	3.9
32	5	30
100	11	60
320	15	114
1000	n. d	304

n. d. = not detectable

7.5.5 Graph Positive Control
Graph for the dose-response relationship of the positive control.



For evaluation of the results, the nominal concentration was used because the difference between real concentration and nominal concentration can be stated as not significant.

7.6 Biological Results 3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctyl acrylate

7.6.1 Determination of the NOEC

The difference between treatment 1000 mg/L and the control can be considered as not significant because the O_2 consumption in the treatment was higher than in the control. Therefore, the concentration 1000 mg/L is stated as NOEC.

The estimated values are presented in the following table.

Table 7.6-a Biological Results Test Item

Parameter	Value	95% Confidence Interval
NOEC	≥ 1000 mg/L	not determinable
3h-EC10	> 1000 mg/L	not determinable
3h-EC50	> 1000 mg/L	not determinable

8 VALIDITY

8.1 EC50 Positive Control

The calculated value for EC50 of the positive control 3,5-dichlorophenole was 7.8 mg/L. A 95% confidence interval of 5.2 – 50 mg/L was calculated.

EC50 of 3,5-dichlorophenole lies within the recommended range of 2 – 25 mg/L.

8.2 Variation in O_2-Consumption of the Controls

Mean oxygen uptake rate was 30.192 mg/(L*h); standard deviation was 1.592 mg/(L*h), corresponding to a coefficient of variation of 5.3 % which is far below the recommended upper limit of 30 %.

8.3 Oxygen Uptake Rate of the Blank Controls

The blank controls oxygen uptake rate was 29.6 mg O_2 per g sludge, which is well above the recommended lower limit of 20 mg O_2 per g of activated sludge in an hour.

9 DISCUSSION

One experiment was performed.

Because volatility of the test item could not be excluded, the experiment was performed in closed Schott flasks without aeration and with analytical determination of the test item in the liquid phase at the beginning and at the end of the test, as described in the OECD guideline. Because of slow dissolution of the test item, only marginal test item concentrations could be measured at the beginning. Surprisingly, no test item could be detected in the highest concentrated treatment at the beginning of the test. This might be caused by insufficient homogenisation of the sample. After three hours stirring, significantly higher test item concentrations could be measured in all treatments.

During validation of the analytical method, the solutions for the determination of recovery and stability in medium were prepared using methanol as solvent. In the main study, no solvent was used because usage of solvents is not described in the guideline. Therefore, due to the slow dissolution of the test item, the actual test item concentrations during the test are unknown. Therefore, the nominal concentrations were used for determination of the results.

At no test item concentration, any inhibitory effect could be observed.

All validity criteria were met. For the estimation of the EC50 of the positive control, the fit showed good statistical correspondence of the data with the dose-response-equation. The positive control gave an EC50 of 7.8 mg/L which is within the recommended range of 2 – 25 mg/L. The coefficient of variation of oxygen uptake rate in control replicates was below 30 % at the end of the test. The oxygen uptake rate of the blank controls was above 20 mg O_2 per gram activated sludge.

The result of the test can be considered valid.

10 DEVIATIONS

10.1 Deviations from the Study Plan

None as known

10.2 Deviations from the Guideline

None as known.

11 RECORDING

One original of study plan and final report, respectively, all raw data of the study and all documents mentioned or referred to in study plan or final report will be kept in the GLP Document Archive of the test facility for fifteen years. After that, the sponsor's instructions will be applied (destruction of documentation). A retain sample of the test item will be kept in the GLP Substance Archive for fifteen years; then, the retain sample will be discarded.

Number of originals which will be sent to the sponsor: 1

12 ANNEX 1: COPY OF GLP-CERTIFICATE

RheinlandPfalz
LANDESAMT FÜR UMWELT, WASSERWIRTSCHAFT UND GEWERBEAUFSICHT

GUTE LABORPRAXIS – GOOD LABORATORY PRACTICE

GLP-BESCHEINIGUNG
STATEMENT OF GLP COMPLIANCE

gemäß/according to § 19b Abs. 1 Chemikaliengesetz

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 2004/9/EG wurde durchgeführt in:

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 2004/9/EC at:

Prüfeinrichtung / Test facility

LAUS GmbH
Auf der Schafweide 20
67489 Kirrweiler

Prüfung nach Kategorien / Areas of Expertise
(gemäß / according ChemVwV-GLP Nr. 5.3/OECD guidance)
1, 3, 4, 5, 6 , 8, 9 (toxikologische In Vitro Prüfungen an Säugerzellen und Bakterien)

Datum der Inspektion / Date of Inspection
(Tag.Monat.Jahr / day.month.year)
29. und 30. November 2010

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

The above mentioned test facility 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 die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.

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

Eine erneute behördliche Überprüfung der Einhaltung der GLP-Grundsätze durch die Prüfeinrichtung ist so rechtzeitig zu beantragen, dass die Folgeinspektion spätestens vier Jahre nach dem Beginn der o.g. Inspektion stattfinden kann. Ohne diesen Antrag wird die Prüfeinrichtung nach Ablauf der Frist aus dem deutschen GLP-Überwachungsprogramm genommen und diese GLP-Bescheinigung verliert ihre Gültigkeit.

Verification of the compliance of the test facility with the Principles of the GLP has to be applied for in time to allow for a follow-up inspection to take place within four years after commencing the above mentioned inspection. Elapsing this term, the test facility will be taken out of the German GLP-Monitoring Programme and this GLP Certificate becomes invalid.

Unterschrift, Datum / Signature, Date

Hill 22.04.11

Dr.-Ing. Stefan Hill - Präsident -
(Name und Funktion der verantwortlichen Person / name and function of responsible person)

Landesamt für Umwelt, Wasserwirtschaft und Gewerbeaufsicht Rheinland-Pfalz

Landesamt für Umwelt, Wasserwirtschaft und Gewerbeaufsicht
Kaiser-Friedrich-Straße 7, 55116 Mainz
(Name und Adresse der GLP-Überwachungsbehörde / Name and adress of the GLP Monitoring Authority)

MESSEN BEWERTEN BERATEN

13 ANNEX 2: ANALYSIS OF DRINKING WATER

Date of analysis: Sep. 2012, Wasserwerk Maikammer

Parameter	Value	Unit
sodium	5.2	mg/L
potassium	2.3	mg/L
calcium	49.7	mg/L
magnesium	6.7	mg/L
aluminium	0.006	mg/L
iron	< 0.005	mg/L
mangane	< 0.005	mg/L
ammonium	< 0.02	mg/L
nitrate	18.0	mg/L
nitrite	< 0.01	mg/L
chloride	14.0	mg/L
sulphate	12.0	mg/L
total organic carbon (TOC)	0.4	mg/L
antimony	< 0.001	mg/L
arsenic	< 0.001	mg/L
lead	< 0.001	mg/L
cadmium	< 0.0002	mg/L
chromium	< 0.001	mg/L
copper	0.002	mg/L
nickel	< 0.002	mg/L
mercury	< 0.0001	mg/L
selenium	< 0.001	mg/L
bor	< 0.02	mg/L
cyanide	< 0.005	mg/L
fluoride	< 0.15	mg/L
benzene	< 0.25	µg/l
polycyclic aromatic hydrocarbons	< 4.0	µg/L
chlorinated organic compounds	< 1.0	µg/L
pesticides and biocides	< LOD	
pH	8.09	
conductivity at 20 °C	330	µS/cm
hardness	1.08	mmol/L

LOD = limit of detection