Section 7.1.2.1.2 Annex Point IIIA XII 2.1	Anaerobic biodegradation	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] Limited exposure [ ]	Technically not feasible [ ] Scientifically unjustified [ ] Other justification [ ]	
Detailed justification:	[redacted]	X
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
Date Evaluation of applicant's justification Conclusion Remarks	[redacted]	

Section A7.1.3 Adsorption / Desorption screening test

Annex Point IIA7.7

Official use only

		1 REFERENCE
1.1	**Reference**	Völkel W (2008); ADSORPTION/DESORPTION OF DECANOIC ACID ON SOILS; RCC Ltd, Itingen; RCC Report No. A86466; Ref nr A7.1.3/01.
1.2	**Data protection**	Yes
1.2.1	Data owner	SOPURA N.V.
1.2.2		
1.2.3	Criteria for data protection	Data on new a.s. for first entry to Annex I/IA / authorisation
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, OECD Guideline 106, EU Directive 98/59/EC C.18
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	03108595700
3.1.2	Specification	As given in section 2
3.1.3	Purity	99%
3.1.4	Further relevant properties	None
3.1.5	Method of analysis	**LC/MS Calibration** Calibration solutions were prepared as follows: **Preliminary test** A stock solution 5 (ST5) was prepared by dissolving 9.67 mg of decanoic acid in 100 mL 9:1 water/acetonitrile (v/v) in a volumetric flask. This resulted in a concentration of 96.7 µg/mL. Stock solution 6 (ST6) was prepared by diluting an aliquot of 5 mL of ST5 in 50 mL 0.01 M $CaCl_2$ solution, resulting in a concentration of 9.67 µg/mL. Thereafter, calibration solutions were prepared with 0.01 M $CaCl_2$ solution as follows:

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Calibration solution	Solution used	Volume solution (µL)	Flask volume (mL)	Final concentration (µg test item/mL)
A	ST6	1200	10	1.16
B	ST6	1000	10	0.98
C	ST6	800	10	0.77
D	ST6	600	10	0.58
E	ST6	400	10	0.39
F	ST6	200	10	0.19
G	ST6	100	10	0.10

Screening Test

The stock solution 7 (ST7) was prepared by dissolving 9.81 mg of decanoic acid in 100 mL 9:1 water/acetonitrile (v/v) in a volumetric flask. This resulted in a concentration of 98.1 µg/mL. Stock solution 8 (ST8) was prepared by diluting an aliquot of 2 mL of ST8 in 20 mL 0.01 M $CaCl_2$ solution, resulting in a concentration of 9.81 µg/mL.

Thereafter, calibration solutions were prepared with 0.01 M $CaCl_2$ solution as follows:

Calibration solution	Solution used	Volume solution (µL)	Flask volume (mL)	Final concentration (µg test item/mL)
A	ST8	1200	10	1.177
B	ST8	1000	10	0.981
C	ST8	800	10	0.785
D	ST8	600	10	0.589
E	ST8	400	10	0.392
F	ST8	200	10	0.196
G	ST8	100	10	0.098
H	ST8	50	10	0.049

Conditions for LC/MS-Analysis

The samples were analysed by High Performance Liquid Chromatography/Mass Spectrometry (LC/MS). After liquid chromatography separation on a reversed phase HPLC column, MS was performed with ESI (Electro Spray Ionisation) in the negative ion SRM (Selected Reaction Monitoring) mode. Typical conditions are given below:

HPLC Conditions (LC/MS53)

Pump: Flux/Rheos-2000
Autosampler: HTCPAL
UV detector: UV 2000 (254 nm)

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Washing solution: Water and methanol

Pre-column: 4 mm x 2 mm, Security guard C-18

Column: 50 mm x 3 mm, 3 µm Phenomenex Luna C-18 (2)

Mobile Phase: Solvent A: 1g/l ammonium carbonate in water
Solvent B: acetonitrile

Sample: A volume of 20 µL was injected

Gradient

Time (min.)	0	3	11	12	12.1	16
Solvent A (%)	90	90	10	10	90	90
Solvent B (%)	10	10	90	90	10	10
Flow [mL/min]	0.5	0.5	0.5	0.5	0.5	0.5

MS Conditions (LC/MS53)

Instrument:	Mass Spectrometer TSQ , Finnigan MAT, San José, USA
Software:	*Xcalibur 1.2* for Windows NT
Ionisation Mode:	Electro Spray Ionisation (ESI)
Detection Mode:	Negative ion detection
Scan Mode:	SRM: m/z171 → m/z171
Sheath Gas Pressure:	Nitrogen, 80 psi
Auxiliary Gas Pressure:	Nitrogen, 10 psi
Capillary temperature:	270°C
Spray Voltage:	4.5 kV

Evaluation of Results

Injected samples were quantified by peak area with reference to the calibration curve. The latter was obtained by correlation of the peak area of the analytical standards with their corresponding concentrations of test item injected (µg/mL), using the following exponential (1) or linear regression (2).

(1) $Y = a \cdot X^b$

or after linearisation

$\ln(Y) = \ln(a) + b \cdot \ln(X)$

where

Y	=	Detector response (peak area)
ln (a)	=	Y-axis intercept (after linearisation)
b	=	Slope (after linearisation)
X	=	Concentration of test item in injected sample

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(µg/mL)

(2) $Y = a + b \cdot X$

where

Y = Detector response (peak area)

a = Y-axis intercept

b = Slope

X = Concentration of test item in injected sample (µg/mL)

The concentration in each sample was calculated by using equation 1 or 2 and the obtained peak area.

The limit of quantification of the test item

The limit of quantification of the test item corresponded to the lowest calibration point used and was set to 0.049 µg/mL.

3.2	**Degradation products**	Degradation products tested: No
3.2.1	Method of analysis for degradation products	N.a.
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	-
3.4	**Soil types**	**Rationale for the Selection of the Test System** **Soils** For this study, five soils differing in particle size, organic matter content, cation exchange capacity and pH were used. The specifications of the soils are given in the table below

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Parameters				Soil I	Soil II	Soil III	Soil IV	Soil V
Site location:				[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Batch:				[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Soil characteristics:								
- pH		(0.01 M $CaCl_2$)		5.7	6.8	6.9	7.2	5.49
- Organic carbon		(g/100 g soil)	%	2.29	1.36	1.9	1.46	2.87
- Cation exchange capacity		(mmol/100g soil)		11.0	4.8	18.0	19.0	26.0
- Nitrogen content			%	0.26	0.13	0.21	0.21	0.35
- Organic matter			%	3.95*	2.34*	3.28*	2.52*	4.95*
- C/N-ratio				8.81*	10.46*	9.05*	6.95*	8.2*
Soil type (according to USDA):				Sandy loam	Loam	Sandy clay	Silt loam	Silty clay
Particle size analyses (mm):								
USDA:								
< 0.002	(clay)	%		7.9	15.0	42.0	16.1	44.3
0.002-0.05	(silt)	%		14.6	36.1	36.1	69.4	53.3
> 0.05	(sand)	%		77.5	48.9	22.0	14.5	2.4

Soil History

Soil I (Speyer 2.2; sandy loam) was sampled by LUFA (Landwirtschaftliche Untersuchungs- und Forschungsanstalt (Rhineland-Palatinate, Germany, 49°18' N, 8°26' E) from the top 20-cm soil layer in June 2006.

Soil II (Mechtildshausen; loam) was sampled by RCC Ltd from the top 20-cm soil layer from an agricultural area in Mechtildshausen (Hesse, Germany, 50° 02' N, 8° 18' E) in July 2005.

Soil III (Speyer 6S; sandy clay) was sampled by LUFA (Landwirtschaftliche Untersuchungs- und Forschungsanstalt in Siebeldingen (Rhineland-Palatinate, Germany, 49°12' N, 8°03' E) from the top 20-cm soil layer in June 2006.

Soil IV (Otzberg; silty loam) was sampled by RCC Ltd from the top 20-cm soil layer from an agricultural area in Otzberg (Hesse, Germany, 49° 49' N, 8° 57' E) in November 2005.

Soil V (Hesingue; silty clay) was sampled by RCC Ltd from the top 20-cm soil layer from an agricultural area in Hesingue (Alsace, France, 47° 34' N, 7° 30' E) in March 2005.

The soils were passed through a 2 mm sieve with minimal force, so that the original texture of the soil was changed as little as possible. The soil moisture content of each soil was determined with three aliquots

3.5 Testing procedure

3.5.1 Test system

Tubes and Equilibration

The study was performed in sealed glass centrifuge tubes. All experiments, including controls (only test item in 0.01 M CaCl2

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solution) and blanks (soil and 0.01 M CaCl2 solution without test item), were performed in duplicate at a constant temperature of 20 ± 2°C. Tubes were shaken in the dark at about 200 rpm. The agitation device kept the soil in suspension during shaking.

Soil Preparation and Conditioning

The air-dried soil samples were equilibrated by shaking with 0.01M $CaCl_2$ solution in tared test tubes. The soil samples were equilibrated with about 90% of the targeted volume of the aqueous phase by shaking overnight (at least 12 hours) before application of the test item.

The pH of the aqueous phase was measured before and after contact with each soil in the preliminary test.

3.5.2 Test solution and Test conditions

Preparation of the Application Solutions

Preliminary test:

The stock solution 1 (ST1) for the preliminary test was prepared by dissolving 9.64 mg of decanoic acid in 100 mL 9:1 water/acetonitrile (v/v) in a volumetric flask. This resulted in a concentration of 96.4 µg/mL. Stock solution 2 (ST2) was prepared by diluting an aliquot of 18 mL of ST1 in 150 mL 0.01 M $CaCl_2$ solution, resulting in a concentration of 11.57 µg/mL. This stock solution (ST2) was used as application solution.

Screening test:

The stock solution 3 (ST3) for the screening test was prepared by dissolving 10.06 mg of decanoic acid in 100 mL 9:1 water/acetonitrile (v/v) in a volumetric flask. This resulted in a concentration of 100.6 µg/mL. Stock solution 4 (ST4) was prepared by diluting an aliquot of 25 mL of ST3 in 200 mL 0.01 M $CaCl_2$ solution, resulting in a concentration of 12.56 µg/mL. This stock solution (ST4) was used as application solution.

Application

After equilibration of the soil with 0.01 M $CaCl_2$ solution, tubes were centrifuged before application for two minutes at about 2800 rpm, then the defined volumes of the corresponding application solutions were added to the surface of the supernatant. Samples were dosed with either 880 µL stock solution ST2 (soil/solution ratio 1:1) or 2200 µL (soil/solution ratio 1:5 and 1:25) (preliminary test). For the screening test samples were dosed with 2000 µL stock solution ST4. Thereafter, the volume of the aqueous solution was adjusted with 0.01 M $CaCl_2$ solution to the target volume for the corresponding soil-to-aqueous solution ratio.

The test tubes were briefly shaken by hand, and then mechanically shaken at about 20°C on a rotary shaker in a temperature-controlled room. The following table summarises the amounts of soil and the volumes of the corresponding 0.01 M $CaCl_2$ solution used.

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Part	Amount of soil [g]	Volume of aqueous phase for equilibration [mL]	Volume of application solution [mL]	Volume* of adsorption solution [mL]	Soil / aqueous phase ratio	Concentration [mg/L 0.01 M $CaCl_2$]
Preliminary test	10	9	0.88	10	1/1	1.0
	5	22.5	2.20	25	1/5	1.0
	1	22.5	2.20	25	1/25	1.0
Screening test	5	22.5	2.00	25	1/5	1.0

* 0.01 M $CaCl_2$ solution was added to bring sample to final volume

Control and Blank Samples

Control samples (duplicate, for each volume of the aqueous phase) with only the test item in 0.01 M $CaCl_2$ solution (no soil) were subjected to precisely the same steps as the test samples, in order to check the stability of the test item in $CaCl_2$ solution and its possible adsorption to the surface of the test vessels.

A blank run (single sample) for each soil type with the same amount of soil and the same total aqueous phase volume (without test item) was subjected to the same test procedure. This served as a background control during the analysis to detect interfering compounds or contaminated soils. The analysis was only performed for the longest interval.

3.6 Test performance

3.6.1 Preliminary test

According to (a)"OECD 106": Yes

Selection of Solid to Liquid Ratio

Two soil types and three soil/solution ratios (six experiments) were used. The soil-to-solution ratios of 1/1, 1/5 and 1/25 were tested

Determination of Adsorption Kinetics Time

Samples were dosed at a rate of about 1 mg/L. After 2, 5, 24 and 48 hours of shaking, duplicate aliquots (individual samples) of the supernatants were measured by LC/MS. Equilibration, centrifugation, etc. were conducted following the procedure previously described (Section 3.1.5).

The stability of the test item in the aqueous phase was determined by LC/MS in the control samples at the same intervals.

3.6.2 Screening test: Adsorption

According to (a)"OECD 106": Yes

Adsorption of the Test Item to Soil:

Following application, the test tubes were shaken horizontally at about 200 rpm at 20±2°C. After shaking, the soils were centrifuged (10 minutes at about 3200 rpm at room temperature) and the supernatants were decanted into tared, labelled sample containers.

The volumes of the supernatants were determined gravimetrically. In the adsorption and desorption test, samples were removed for LC/MS analysis at different shaking intervals (after centrifugation).

The equilibrium concentration of the test item, and its total amount in the aqueous phase, were calculated based on the results of the HPLC

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analyses. The amount of test item adsorbed onto soil particles was obtained from the difference between the initial and final amount of test item in the aqueous phase. Only for the mass balance tubes, the amount of decanoic acid adsorbed to soil was additionally quantitatively analysed by extraction and HPLC analysis.

3.6.3 Screening test: Desorption

According to (a)"OECD 106": Performed

Desorption of the Test Item:

After 4 hours of adsorption in the screening test, the supernatant was decanted after centrifugation. The same volume of 0.01 M $CaCl_2$ as used for the adsorption was added back to the soil pellet. Thereafter, desorption mixtures were agitated in the same manner as for the adsorption step. Samples were then centrifuged and the supernatant analysed by LC/MS, after either 2, 5, and 24 hours of shaking.

3.6.4 HPLC-method

According to (a)" OECD-HPLC-method"[1]: Yes

HPLC Conditions (LC/MS53)

Pump: Flux/Rheos-2000
Autosampler: HTCPAL
UV detector: UV 2000 (254 nm)
Washing solution: Water and methanol
Pre-column: 4 mm x 2 mm, Security guard C-18
Column: 50 mm x 3 mm, 3 µm Phenomenex Luna C-18 (2)
Mobile Phase: Solvent A: 1g/l ammonium carbonate in water
Solvent B: acetonitrile
Sample: A volume of 20 µL was injected

Gradient :

Time (min.)	0	3	11	12	12.1	16
Solvent A (%)	90	90	10	10	90	90
Solvent B (%)	10	10	90	90	10	10
Flow [mL/min]	0.5	0.5	0.5	0.5	0.5	0.5

3.6.5 Other test

Sterilisation of the soils by sodium azide solution, autoclaving, heating above 100°C, irradiation or gamma irradiation were investigated to prevent degradation of decanoic acid (see 4 Results for details).

4 RESULTS

4.1 Preliminary test

All values reported here represent the mean of duplicate sample analyses.

In the preliminary test, the equilibration time for the adsorption was tested for two soils at three soil-to-aqueous solution ratios (1/1, 1/5 and 1/25) at a concentration of 1 mg/L. Virtually all of the test item had disappeared from the supernatant solutions after 24 and 48 hours of adsorption for both soils, thereby indicating complete adsorption. The

[1] OECD (1999) OECD-Guidelines for the Testing of Chemicals. Proposal for a new guideline 121: Estimation of the adsorption coefficient (K_{OC}) on soil and on sewage sludge using High Performance Liquid Chromatography (HPLC), Draft Document (August 1999).

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same behaviour was observed in the control samples (without soil): No test item was found in the supernatant of one sample after 48 hours.

It was therefore necessary to determine the reason for the test item disappearing from solution:

a. It was strongly adsorbed to soil. Based on the water solubility of decanoic acid (0.15 g/L), an extremely strong adsorption could not be expected.

b. The test item disappeared from the supernatant due to complexation with Ca-ions. Since all calibration solutions for LC/MS analysis were prepared in 0.01M $CaCl_2$ solution and no disappearance of the test item was observed, this possibility was ruled out.

c. Degradation of the test item. Since a large variation in the results was observed for some samples, rapid degradation of the test item was assumed.

Blank samples showed only small signals, i.e. below the lowest reference solution, at the retention time of the test item.

Since measurable concentrations of the test item were still observed after 5 hours of adsorption, and the ratio 1/5 gave moderate adsorption, a screening test was performed using five sterilised soils, a ratio of 1/5 and an adsorption time of 4 hours

4.2 Screening test: Adsorption

For the sterilisation of soil, different methods are possible such as treatment with sodium azide solution, autoclaving, heating above 100°C or irradiation. Since sterilisation should not change the soil structure nor influence the adsorption, gamma-irradiation was the chosen method to sterilise the five soils used in the screening test. Additionally, the $CaCl_2$ solution was autoclaved.

Even under sterile conditions the test item completely disappeared from the supernatant of soils I, IV and V and in Sample B of soil II (Table 3). Only in soil III (type: sandy clay), the expected low adsorption of the test item was observed. Therefore an accurate Koc value could not be calculated.

In the sterile control solutions (without soil), the test item was recovered

The mass balance was carried in the course of the adsorption test for all five soils. After 4 hours of adsorption, the supernatant was removed and the remaining soil was extracted three times using acetonitrile/water (4:1; (v/v)), in order to show the extractability of the test item. The resulting extracts were combined and the test item quantified by LC/MS. Virtually no test item could be detected with amounts of decanoic acid below or close to the detection limit.

Blank extracts in 0.01 M $CaCl_2$ solution from untreated soils were therefore spiked with decanoic acid at a concentration of 1.0 mg/L and analysed by LC/MS. Again, no test item could be detected. This showed that even without direct contact to soil, decanoic acid degraded in the soil extracts. The results of calibration solutions in 0.01 M $CaCl_2$ run before, midway and at the end of a series of samples showed no degradation of the test item and therefore the reliability of the analytical method.

Complete recovery of the test item was only possible after spiking blank extracts, which had been sterilised by autoclaving.

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Since sterilisation of the soil by gamma-irradiation proved insufficient (for example fungi spores could survive and cause the degradation of decanoic acid), a more harsh method of sterilisation of the soil was used, heating to 120°C. Using this method, higher concentrations of decanoic acid in the supernatants could be measured in several samples after four hours of adsorption. However, degradation could be not be avoided for all samples.

In order to eliminate the fungi spores or microorganisms responsible for the degradation of decanoic acid in the presence of soil, it would be necessary to heat the soil above 120°C, for example to 150°C. Heating to 150°C would make it necessary to demonstrate that the soil structure remains intact after such a treatment.

For the above-mentioned reasons, no Koc value could be calculated for decanoic acid.

4.3 Screening test: Desorption

Desorption was performed for the same five soils at a test concentration of 1.0 mg/L and at a soil-to-solution ratio of 1/5 after 4 hours of adsorption. The supernatants were analysed after 2, 4 and 24 hours of desorption.

No decanoic acid could be detected in desorption solutions.

Calculations

4.3.1	Ka , Kd	Not possible to calculate
4.3.2	Ka_{oc} , Kd_{oc}	Not possible to calculate

Degradation product(s) None found

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The adsorption and desorption behaviour of Decanoic acid were determined in the following five soils using the batch equilibrium method according to the OECD Guideline no. 106: Speyer 2.2 (soil I, sandy loam), Mechtildshausen (soil II, loam), Speyer 6S (soil III, sandy clay), Otzberg (soil IV, silt loam) and Hesingue (soil V, silty clay).

The distribution of a chemical between soil and aqueous phases is a complex process depending on a number of different factors such as the chemical nature of the substance, the characteristics of the soil and climatic factors. The batch equilibrium method attempts to define the numerous phenomena and mechanisms involved in the process of adsorption of a chemical by soil, and more importantly, it provides valuable information on the environmental relevance of the adsorption of a chemical.

The purpose of this study was to determine the sorption of the test item, decanoic acid, to soils of differing pH, organic carbon content and textural classification.

The study followed closely the OECD 106 guideline.

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5.2 Results and discussion

From available other studies it is known that decanoic acid is poorly soluble in water. Further it was found to be readily biodegradable. Moreover decanoic acid is an intermediate in the tricarboxylic acid pathways, that metabolic path is present in almost all living species and cells because finally it leads to the synthesis of triglycerides of fatty acids building the cell membrane. Vapour pressure and therefore volatility is low. Based on these properties the results observed are fully in line with the properties of decanoic acid.

Initially a preliminary test was conducted using soils I (Sandy loam) and soil II (loam), with three soil-to-solution ratios (1/1, 1/5 and 1/25) at a test concentration of about 1 mg/L and up to 48 hours adsorption time. The soil-to-aqueous phase suspensions were incubated in sealed glass centrifuge tubes on a shaker at about 150 strokes per minute in the dark at 20 ±2°C. After 2, 5, 24, and 48 hours of agitation, aliquots of the aqueous phase were analysed by LC/MS.

No test item was detected in the supernatants of all soils and ratios, nor in the controls. Since strong adsorption of the test item to soil and complexation of the test item with Ca-ions were not possible, it was assumed that the test item degraded under the test conditions.

Additional efforts were therefore made to minimise degradation of the test item. In the subsequent screening test, soil sterilised by gamma-irradiation was used together with sterile $CaCl_2$ solution. Except for soil III (Sandy clay), only in the absence of soil i.e. in sterile control solutions, could the test item be recovered.

A more severe sterilisation method was therefore used (soil heated to 120°C). The results showed higher concentrations of decanoic acid in the supernatants in most of the samples after four hours of adsorption. However, degradation could be not be avoided for all samples.

Even in blank extracts in 0.01 M $CaCl_2$ solution from untreated soils spiked with decanoic acid at a concentration of 1.0 mg/L, no test item could be detected. Therefore the test item degraded even without direct contact with soil. In mass balance samples, virtually no test item could be detected with amounts of decanoic acid below or close to the detection limit.

Complete recovery of the test item was only possible after spiking blank extracts which had been sterilised by autoclaving

In order to eliminate the fungi spores or microorganisms responsible for the degradation of decanoic acid in the presence of soil, it would be necessary to heat the soil above 120°C, for example to 150°C. Heating to 150°C would make it necessary to demonstrate that the soil structure remains intact after such a treatment.

For the above-mentioned reasons, no Koc value could be calculated for decanoic acid.

5.2.1	Adsorbed a.s. [%]	See results and discussion
5.2.2	K_a	See results and discussion
5.2.3	K_d	See results and discussion
5.2.4	Ka_{oc}	See results and discussion

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5.2.5	Ka/Kd	See results and discussion
5.2.6	Degradation products (% of a.s.)	None
5.3	**Conclusion**	The test was preformed in full agreement with the OECD Guideline106. However, despite great efforts to sterilise the soils no decanoic acid was detected and because other causes have been excluded it was concluded that the test item degraded under the test conditions. For the above-mentioned reasons, no Koc value could be calculated. For the purpose of assessing the risk to soil organisms and leakage of decanoic acid to groundwater the study is applicable because the results show that there is a negligible likelihood that decanoic acid would present after a short time.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
Date **Materials and Methods** **Results and discussion** **Conclusion** **Reliability** **Acceptability**	[redacted]
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_1 _3-1: Classification and physico-chemical properties of soils used as adsorbents

	Soil 1	Soil 2	Soil 3	Soil 4	Soil 5
Soil order	-	-	-	-	-
Soil series	F2.22305	07/05	F6S2305	11/05	03/05
Classification	Sandy loam	Loam	Sandy clay	Silt loam	Silty clay
Location	Speyer 2.2	Mechtildshausen	Speyer 6S	Otzberg	Hesingue
Horizon	-	-	-	-	-
Sand [%]	77.5	48.9	22.0	14.5	2.4
Silt [%]	14.6	36.1	36.1	69.4	53.3
Clay [%]	7.9	15.0	42.0	16.1	44.3
Organic carbon [%]	2.29	1.36	1.9	1.46	2.87
Carbonate as $CaCO_3$	-	-	-	-	-
insoluble carbonates [%]	-	-	-	-	-
pH (0.01 $McaCl_2$)	5.7	6.8	6.9	7.2	5.49
Cation exchange capacity (MEQ/100 g)	11.0	4.8	18.0	19.0	26.0
Extractable cations (MEQ/100 g)	-	-	-	-	-
Ca	-	-	-	-	-
Mg	-	-	-	-	-
Na	-	-	-	-	-
K	-	-	-	-	-
H	-	-	-	-	-
Special chemical/mineralogical features	-	-	-	-	-
Clay fraction mineralogy	-	-	-	-	-

Table A7_1 _3-2: Results of preliminary test:

Test substance	Decanoic acid
Sample purity	99%
Weighed soil	
Volume of $CaCl_2$ solution	
Nominal concentration of a.s. final solution	0.10 – 1.16 mg/L
Analytical concentration final of a.s. solution	–
Concentration of the test solution (show calculation)	
Details of the analytical method used:	
Method	LC/MS-Analysis
Recovery rate	-
Detection limit	0.05 mg/L

Table A7_1 _3-3: Results of screening test - adsorption: *Not applicable see text*

	Soil 1		Soil 2		Soil 3		Soil 4		Soil 5	
Concentration of test material [mg/l]										
After contact of....hours with soil										
Correction for blank with soil										
Correction for blank without soil										
Final corrected concentration [mg/l]										
Initial concentration of test solution [mg/l]										
Decrease in concentration [mg/l]										
Quantity adsorbed [µg]										
Quantity of soil [g of oven-dried equivalent]										
Quantity adsorbed [µg] per gram of soil										
Test material adsorbed [%]										
Temperature [°C]										
Volume of solution recovered after centrifugation [ml]										
Volume of solution not recovered [ml]										
Corresponding quantity of test substance [mg]										

Table A7_1 _3-4: Results of screening test - desorption: *Not applicable see text*

	Soil 1		Soil 2		Soil 3		Soil 4		Sil4	
Temperature [°C]										
Concentration in combined washings [mg/l]										
Corresponding quantity of test material [mg]										
Quantity desorbed [µg]										
[%] of adsorbed test material, which is desorbed										
[%] of adsorbed test material, which is not desorbed										

Section A7.3.1 Annex Point III A7.5	Phototransformation in air (estimation method)	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] Limited exposure [X]	Technically not feasible [ ] Scientifically unjustified [ ] Other justification [X]	
Detailed justification:	[redacted]	
Undertaking of intended data submission [ ]		

Evaluation by Competent Authorities	
Date	[redacted]
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A 7.4.1.1 Annex Point IIA7.2	Acute toxicity to fish	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] Limited exposure [ ]	Technically not feasible [ ] Scientifically unjustified [ ] Other justification [ ]	
Detailed justification:	[redacted]	

Section A 7.4.1.1 **Acute toxicity to fish**
Annex Point IIA7.2

[redacted]

Section A 7.4.1.1 Annex Point IIA7.2	Acute toxicity to fish
	[redacted]
Undertaking of intended data submission [ ]	
	Evaluation by Competent Authorities
Date	[redacted]
Evaluation of applicant's justification	
Conclusion	
Remarks	-

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1

[redacted]

			Official use only
1.1	**Reference**	[redacted]	
1.2	**Data protection**		
1.2.1	Data owner		
1.2.2			
1.2.3	Criteria for data protection		
2.1	**Guideline study**		
2.2	**GLP**		
2.3	**Deviations**		
3.1	**Test material**		
3.1.1	Lot/Batch number		
3.1.2	Specification		
3.1.3	Purity		
3.1.4	Composition of Product		
3.1.5	Further relevant properties		
3.1.6	Method of analysis		

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1

[redacted]

[redacted]

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1 [redacted]

[redacted]

3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	x
3.3	**Reference substance**	
3.3.1	Method of analysis for reference substance	
3.4	**Testing procedure**	
3.4.1	Dilution water	

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1 [redacted]

3.4.2	Test organisms	[redacted]
3.4.3	Test system	
3.4.4	Test conditions	
3.4.5	Duration of the test	
3.4.6	Test parameter	
3.4.7	Sampling	
3.4.8	Monitoring of TS concentration	
3.4.9	Statistics	
Limit Test		
4.1.1	Concentration	
4.1.2	Number/ percentage of	

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1 [redacted]

	animals showing adverse effects	[redacted]
4.1.3	Nature of adverse effects	
Results test substance		
4.1.4	Initial concentrations of test substance	

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1 [redacted]

4.1.5 Actual concentrations of test substance

[redacted]

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1 [redacted]

4.1.6 Effect data (Mortality)

4.1.7 Concentration / response curve

4.1.8 Other effects

Results of controls

4.1.9 Number/ percentage of animals showing adverse effects

4.1.10 Nature of adverse effects

Test with reference substance

[redacted]

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1 [redacted]

4.1.11 Concentrations

4.1.12 Results

5.1 Materials and methods

[redacted]

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1 [redacted]

[redacted]

5.2 Results and discussion

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1

[redacted]

[redacted]

x

5.2.1 LC_0 x

5.2.2 LC_{50} x

5.2.3 LC_{100} x

5.3 Conclusion

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1 [redacted]

[redacted]

x
x

5.3.1 Other Conclusions
5.3.2 Reliability
5.3.3 Deficiencies

Evaluation by Competent Authorities

[redacted]

Date

Materials and Methods

Results and discussion

Conclusion

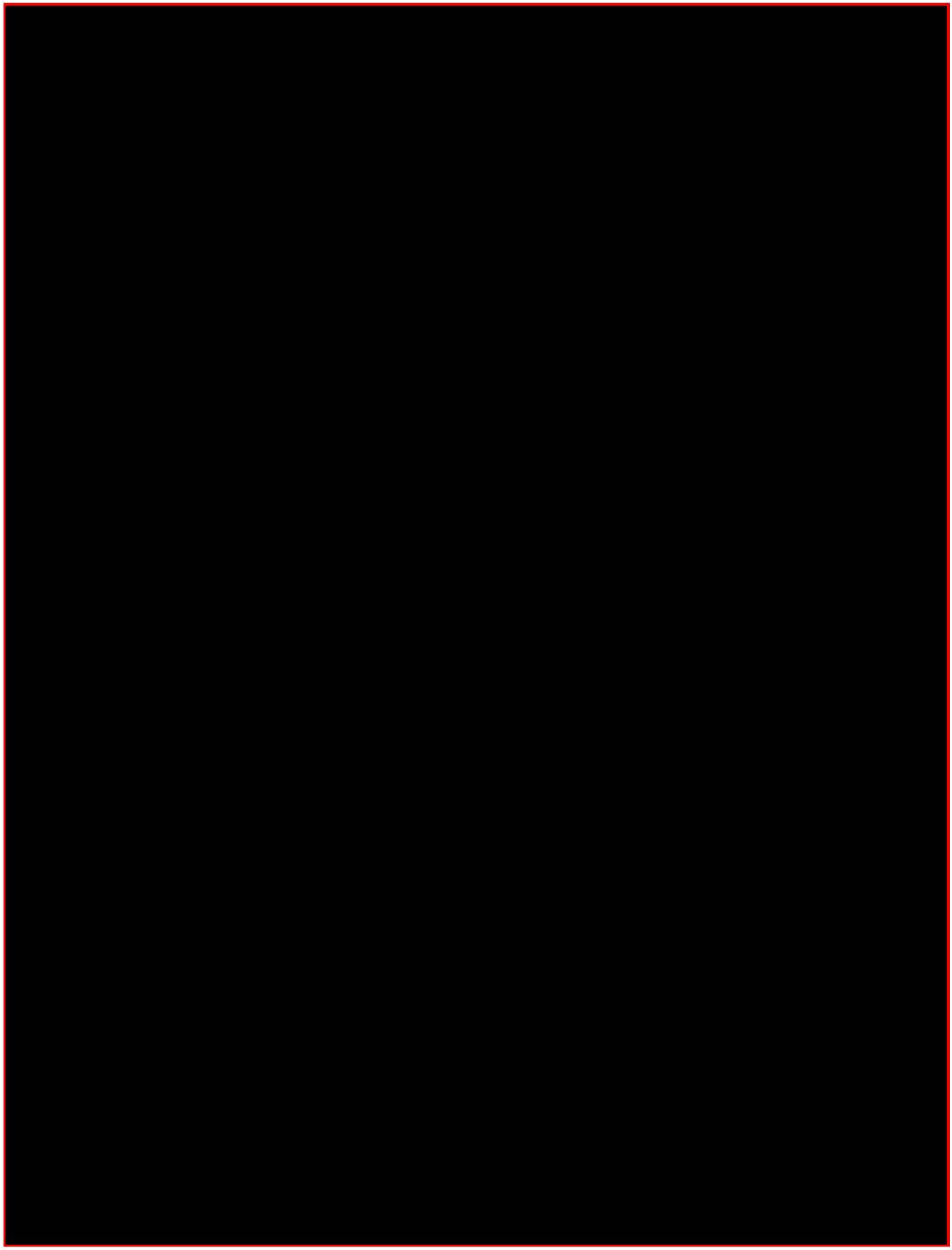
Reliability

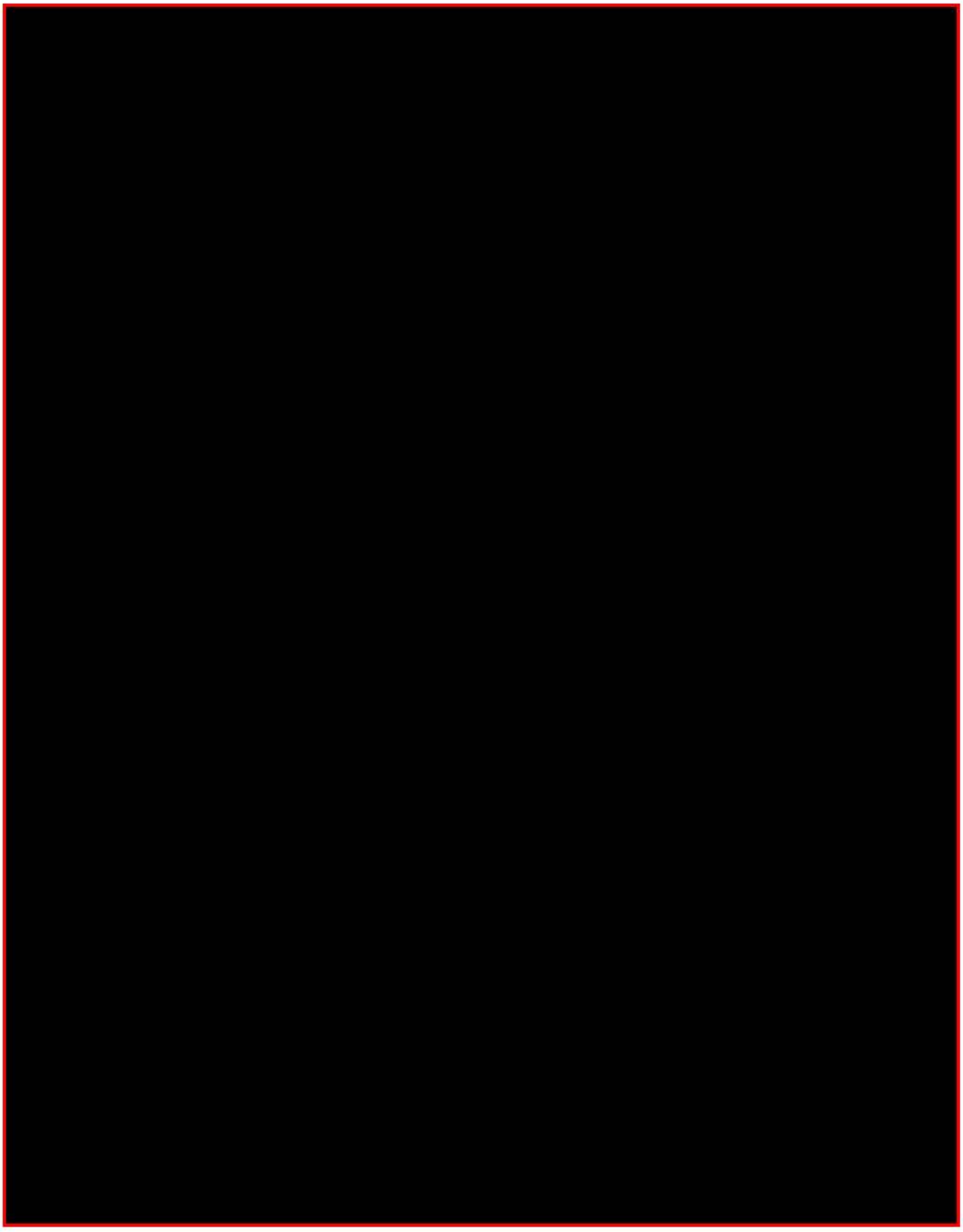
Acceptability

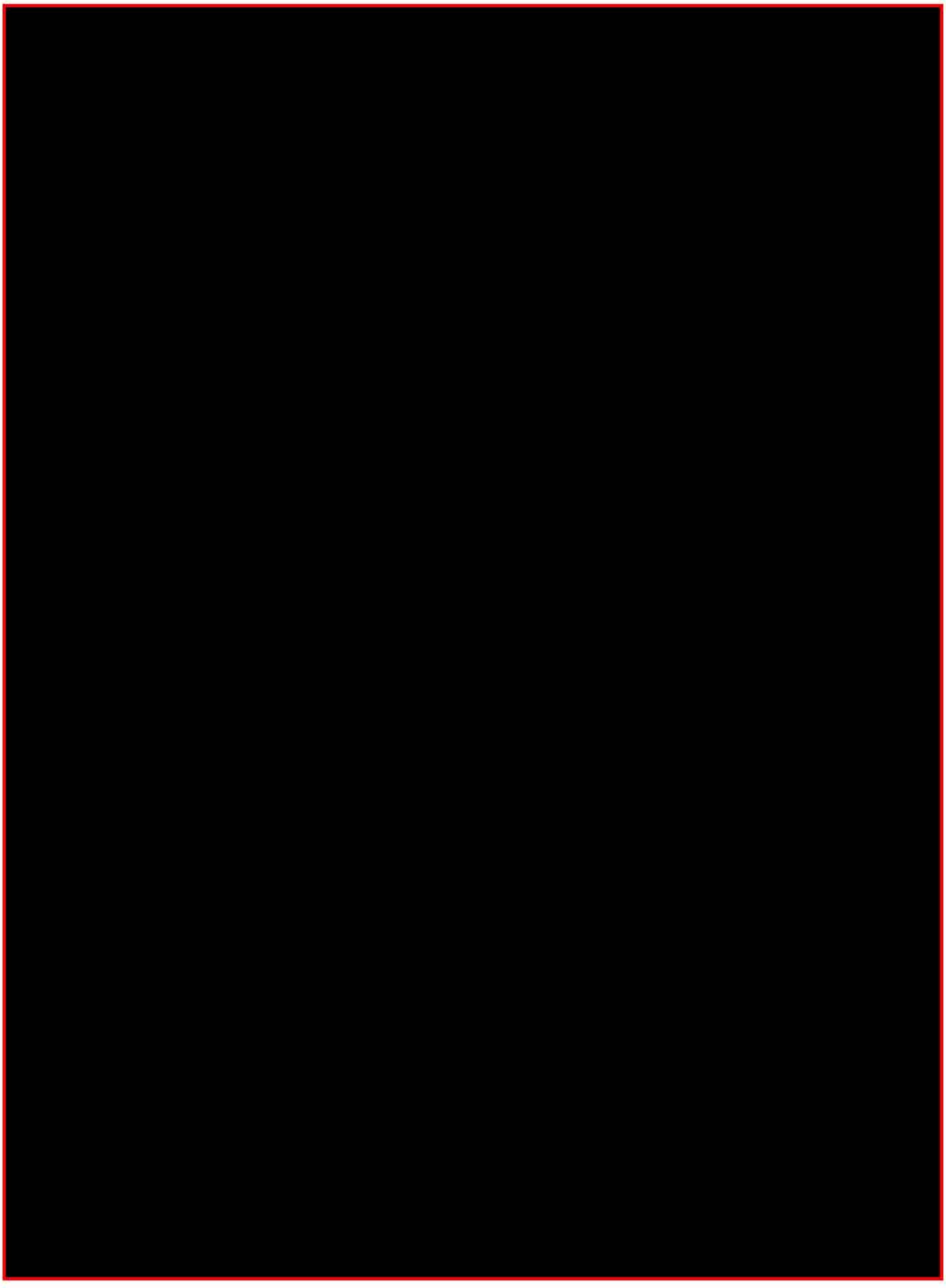
Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1 [redacted]

Remarks	-







Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 *Specify species, e.g. Daphnia magna*

			Official use only
		1 REFERENCE	
1.1	**Reference**	Bätscher Roger (2006); DECANOIC ACID: ACUTE TOXICITY TO *DAPHNIA MAGNA* IN A 48-HOUR IMMOBILIZATION TEST; RCC Ltd, Itingen, Switzerland; RCC Study Number: A86488; Ref nr A.7.4.1.2/01	
1.2	**Data protection**	Yes	
1.2.1	Data owner	SOPURA N.V.	
1.2.2			
1.2.3	Criteria for data protection	Data on exisitng a.s. submitted for first time for entry to Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes; OECD Guideline No. 202, EU Commission Directive 92/69/EEC, C.2	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Decanoic acid. As given in section 2	
3.1.1	Lot/Batch number	031 08595700	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	99.7%	
3.1.4	Composition of Product	N.a.	
3.1.5	Further relevant properties	Poorly soluble substance	x
3.1.6	Method of analysis	The applied analytical method is gas chromatography. The samples were stored deep-frozen and protected from light until analysis was performed. GC conditions	

Section A7.4.1.2 Acute toxicity to invertebrates

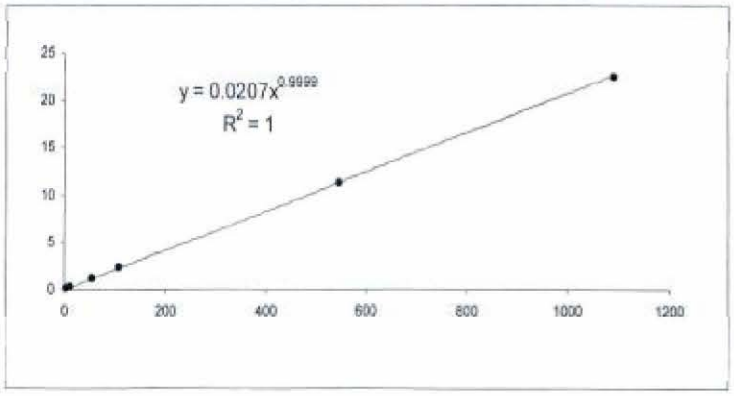
Annex Point IIA7.2 *Specify species, e.g. Daphnia magna*

Gas chromatograph:	AGILENT 6890
Auto sampler:	HP 7673A
Injection Mode:	Pulsed Splitless Injection Volume: 1 µL 4 mm ID Splitless Insert: Glasswool
Inlet Purge Time:	0.6 Minute Gas Saver On at 2 Minutes
Column:	DB WAX (40 m; 0.32 mm inner diameter; 0.50 µm film thickness)
Carrier gas:	Helium Column Flow: 2.5 mL/Minute Purge Flow: 50 mL/Minute Septum Purge: Constant
Temperature program:	Injector: 250°C Oven: 60°C, for 1 Minute with 15°C/Minute to 250°C, for 5 Minutes
Detector:	FID
Detector gas (mL/min)	H_2 30; Air 400; N_2 30
Retention time:	Decanoic acid: approx. 11.3 Minutes Octanoic acid (Internal standard): approx. 9.95 Minutes

Example of calibration data of test item standards

Standard [mg/L]	Peak area measured [counts]	Peak area Internal standard measured [counts]	Deviation from calculated value [%]
5.46	20.6	182	0.1
10.9	41.2	183	-0.6
54.6	213	188	0.3
109	425	187	0.5
546	2187	193	0.3
1091	4294	191	-0.6

Calibration graph and equation.



Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 *Specify species, e.g. Daphnia magna*

3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Prior to the study, a pre-experiment without GLP was performed to investigate the solubility of the test item in the test water by analysis. In this pre-experiment, the test item was completely dissolved in the test water after stirring for 24 hours. Before the start of the test and prior to the test medium renewal, the test medium of the highest nominal concentration of 100 mg/L was prepared by dissolving 150.3 and 149.9 mg of test item completely in 1500 mL of test water using ultrasonic treatment for 20 minutes and intense stirring for 24 hours at room temperature in the dark. The stirring time of 24 hours was chosen based on the results of the pre-experiment mentioned above.	x
3.3	**Reference substance**	Positive control: Potassium dichromate; preformed once a year showing that the sensitivity of the test organisms was within the historical range of the RCC laboratory (48-hour EC50 from 1996 to 2006: 0.53-1.1 mg/L).	
3.3.1	Method of analysis for reference substance	N.a.	
3.4	**Testing procedure**		
3.4.1	Dilution water	Reconstituted test water: analytical grade salts were dissolved in purified water to obtain the following nominal concentrations: $CaCl_2 \times 2H_2O$: 2.0 mmol/L (= 294 mg/L) $MgSO_4 \times 7H_2O$: 0.5 mmol/L (= 123 mg/L) $NaHCO_3$: 0.75 mmol/L (= 65 mg/L) KCl : 0.075 mmol/L (= 5.8 mg/L) Water Hardness : 2.5 mmol/L (= 250 mg/L as $CaCO_3$) Alkalinity : 0.8 mmol/L Ratio of Ca : Mg = 4 : 1 (based on molarity) Na : K = 10 : 1 (based on molarity) The test water was aerated prior to the start of the study until oxygen saturation was reached. During the test period, the test water was not aerated.	
3.4.2	Test organisms	*Daphnia magna* Straus breed in house by RCC Ltd.	
3.4.3	Test system	The study was performed with young daphnids of a clone of the species *Daphnia magna* Straus. A clone of this species (defined by the supplier as clone 5) was originally supplied by the University of Sheffield/UK in 1992. Since that time, the clone has been bred in the laboratories of RCC in reconstituted water of the quality identical to the water quality used in the tests (in respect to pH, main ions, and total hardness) and under temperature and light conditions identical to those of the tests (see below). At the start of the test, the organisms used in the test were 6-24 hours old and were not first brood progeny.	
3.4.4	Test conditions	*Give relevant test conditions in tabular form (see table A7_4_1_2-5)* A semi-static test with test medium renewal after 24 hours was	

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 *Specify species, e.g. Daphnia magna*

		performed to keep the concentrations of the test item in the test media as constant as possible during the test period of 48 hours. After 24 hours the test organisms were placed in clean test vessels with freshly prepared test medium of the corresponding concentration. The test was performed under the following conditions: Water temperature: 20 - 21 °C during the test period (Table 5). The test was performed in a temperature-controlled room (room temperature continuously monitored). Light conditions: A 16-hour light to 8-hour dark photoperiod (with a 30 minute transition period). Light intensity during the light period was between approximately 470 and 640 Lux. Test duration: 48 hours
3.4.5	Duration of the test	48 hours
3.4.6	Test parameter	Immobility The immobility of the daphnids was determined by visual inspection after 24 and 48 hours of exposure. Those daphnids that were not able to swim within 15 seconds after gentle agitation of the test vessel were considered to be immobilized. The 24-hour and 48-hour EC50 and the 95% confidence limits were calculated by Moving Average Interpolation (THOMPSON, W.R., WEIL, C.S. (1952): On the Construction of Tables for Moving Average Interpolation, Biometries 8, 51-54; FINNEY, DJ. (1978): Statistical Methods in Biological Assay, 3rd Edition, Charles Griffin, London).
3.4.7	Sampling	For the determination of the actual test item concentrations, duplicate samples were taken from the freshly prepared test media of all test concentrations and the control at the start of the test and at the test medium renewal after 24 hours. To confirm the maintenance of the test item concentrations over the test medium renewal period of 24 hours, duplicate samples were taken from the aged test media and the control at the end of both test medium renewal periods (stability samples). For sampling of the stability samples, the contents of the respective replicates were combined prior to sampling. All samples were deep-frozen (at about -20 °C) immediately after sampling. In preexperiments for investigation of the storage stability (without GLP), the test item was found to be stable in the test water under these storage conditions. The concentrations of the test item Decanoic acid were analytically measured in the duplicate test media samples from the nominal concentrations of 10 mg/L (48-hour NOEC) and 100 mg/L (highest test concentration) from all sampling times. From the control, one of the duplicate samples was analyzed from each of the sampling times.
3.4.8	Monitoring of TS concentration	Yes 24 hours
3.4.9	Statistics	The 95% confidence limits were calculated by Moving .Average

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 *Specify species, e.g. Daphnia magna*

		Interpolation
		4 RESULTS
Limit Test		Not performed
4.1.1	Concentration	-
4.1.2	Number/ percentage of animals showing adverse effects	-
4.1.3	Nature of adverse effects	-
Results test substance		
4.1.4	Initial concentrations of test substance	2.2, 4.6, 10, 22, 46, 100 mg/L. The pH of the test media and the control was adjusted to approximately 6.5-6.7 to lie within the physiological range for the daphnids.
4.1.5	Actual concentrations of test substance	At the start and the end of the test medium renewal periods, the analytically determined concentrations of the test item in the analyzed test media were between 88 and 97% of the nominal values. Thus, the test item Decanoic acid was stable during the test medium renewal period of 24 hours under the conditions of the test. The reported biological results were based on the nominal concentrations of the test item.
4.1.6	Effect data (Immobilisation)	24-hour EC50: 24 mg/L; 95% confidence interval: 19-30 mg/L 24-hour EC0: 10 mg/L 24-hour EC100: 46 mg/L 48-hour EC50: 16 mg/L; 95% confidence interval: 14-19 mg/L 48-hour EC0: 10 mg/L; 48-hour NOEC 48-hour EC100: 46 mg/L;
4.1.7	Concentration / response curve	Not calculated.
4.1.8	Other effects	None
Results of controls		Not reported. Historical data available
Test with reference substance		Not performed
4.1.9	Concentrations	-
4.1.10	Results	-
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The acute toxicity of the test item Decanoic acid to Daphnia magna was determined in a 48-hour static test according to the EU Commission Directive 92/69/EEC, Part C.2 (1992) and the OECD Guideline for Testing of Chemicals, No. 202 (2004).

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 *Specify species, e.g. Daphnia magna*

The test was performed in a semi-static procedure with a renewal of the test media after 24 hours. The analytically determined concentration were > 80% and therefore classify the concentration as stable and allowing the use of nominal concentrations.

The endpoint for toxicological determination was immobility of the daphnids.

5.2 Results and discussion

There were no deviations from the protocol or the guidelines.

Decanoic acid is a poorly soluble substance. The solubility has been determined before the study and was found to be sufficient for the purpose of the study.

The pH of the test media was adjusted to 6.5-6.7 to lie within the physiological range of daphnids. As the analytical determined concentration were between 88 and 97% the nominal value as used.

The observations are as can be expected for this type of test and do not show any reportable observation outside the protocol.

5.2.1	EC_0	10 mg/L
5.2.2	EC_{50}	16 mg/L; 95% confidence interval: 14-19 mg/L
5.2.3	EC_{100}	46 mg/L

5.3 Conclusion

The acute toxicity of the test item Decanoic acid to Daphnia magna was determined in a 48-hour static test according to the EU Commission Directive 92/69/EEC, Part C.2 (1992) and the OECD Guideline for Testing of Chemicals, No. 202 (2004).

Since there are no derivations from the guideline, the concentrations of the test item was stable, no adverse observation are made the study is valid without restrictions.

The EC_{50} is 16 mg/L and NOEC is 10 mg/L.

5.3.1	Other conclusions	-
5.3.2	Reliability	1
5.3.3	Deficiencies	No

Evaluation by Competent Authorities

Date

Materials and Methods

[redacted]

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 *Specify species, e.g. Daphnia magna*

Results and discussion	[redacted]
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7_4_1_2-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes/No
Vehicle	No
Concentration of vehicle	N.a.
Vehicle control performed	N.a.
Other procedures	Semi-static procedure with renewal after 24 hours

Table A7_4_1_2-2: Dilution water

Criteria	Details
Source	Reconstituted test water: analytical grade salts were dissolved in purified water
Alkalinity	0.8 mmol/L
Hardness	2.5 mmol/L (= 250 mg/L as $CaCO_3$)
pH	Adjusted to 6.5-6.7 the physiological range for daphnids.
Ca / Mg ratio	4 : 1 (based on molarity)
Na / K ratio	10 : 1 (based on molarity)
Oxygen content	8.7 – 9.0 mg/L
Conductance	Not reported
Holding water different from dilution water	No

Table A7_4_1_2-3: Test organisms

Criteria	Details
Strain	*Daphnia magna* Strauss
Source	RCC Ltd.
Age	6-24 hours old
Breeding method	RCC SOP
Kind of food	Not reported
Amount of food	Not reported
Feeding frequency	Not reported
Pretreatment	The daphnia are bred under similar conditions as applied during the test, but with feeding.
Feeding of animals during test	No

Table A7_4_1_2-4: Test system

Criteria	Details
Renewal of test solution	in case of renewal, describe intervals and procedure
Volume of test vessels	100 mL glass beakers filled with 50 mL test medium
Volume/animal	10 mL per daphnia
Number of animals/vessel	5
Number of vessels/ concentration	4
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_2-5: Test conditions

Criteria	Details
Test temperature	Table 5: Temperature of the test media and the plant(s)

Nominal test item concentration	Exposure time			
	0 h	24 h		48 h
(mg/L)	new	o.c	new	o.c
Control	20	20	21	20
2.2	20	20	21	20
4.6	20	20	21	20
10	20	20	21	20
22	20	20	21	20
46	20	20	21	20
100	20	20	21	20

Dissolved oxygen	Table 5: Dissolved oxygen concentrations in the test media and the control Nominal test item concentration (mg/L) / Exposure time: 0 h (new), 24 h (old), 24 h (new), 48 h (old) Control: 8.9 / 8.8 / 9.0 / 8.6 2.2: 8.9 / 8.8 / 8.9 / 8.8 4.6: 8.9 / 8.8 / 9.0 / 8.8 10: 8.8 / 8.7 / 9.0 / 8.8 22: 8.8 / 8.7 / 8.9 / 8.8 46: 8.8 / 8.8 / 8.9 / 8.5 100: 8.8 / 8.5 / 8.9 / 8.5
pH	Table 4: pH values in the test media and the control (after adjustment of pH, see Section 2.5.2) Nominal test item concentration (mg/L) / Exposure time: 0 h (new), 24 h (old), 24 h (new), 48 h (old) Control: 8.8 / 8.8 / 8.5 / 8.6 2.2: 8.8 / 8.8 / 8.8 / 8.6 4.6: 8.7 / 8.6 / 8.8 / 8.8 10: 8.8 / 8.6 / 8.8 / 8.7 22: 8.8 / 8.6 / 8.8 / 8.7 46: 8.8 / 8.8 / 8.8 / 8.7 100: 8.8 / 8.8 / 8.8 / 8.8
Adjustment of pH	Yes 6.5-6.7
Aeration of dilution water	No
Quality/Intensity of irradiation	Light intensity during the light period was between approximately 470 and 640 Lux.
Photoperiod	A 16-hour light to 8-hour dark photoperiod (with a 30 minute transition period).

Table A7_4_1_2-6: Immobilisation data

Test-Substance Concentration (nominal/effective)[1] [mg/l]	Immobile *Daphnia*						
	Number		Percentage		Oxygen [mg/l]	pH	Tempera-ture [°C]
	24 h	48 h	24 h	48 h	48 h	48 h	48 h
0	0	0	0	0	8.6	6.5	20
2.2	0	0	0	0	8.8	6.6	20
4.6	0	0	0	0	8.8	6.6	20
10	0	0	0	0	8.6	6.7	20
22	7	17	35	85	8.6	6.7	20
46	20	20	100	100	8.5	6.7	20
100	20	20	100	100	8.5	6.8	20

[1] specify, if TS concentrations were nominal or measured; measured concentration >80%

Table A7_4_1_2-7: Effect data

	EC_{50}[1]	95 % c.l.	EC_0[1]	EC_{100}[1]
24 h [mg/l]	24 (n)	19-30 mg/L	10 (n)	46 (n)
48 h [mg/l]	16 (n)	14-19 mg/L	10 (n)	46 (n)

[1] indicate if effect data are based on nominal (n) or measured (m) concentrations

Table A7_4_1_2-8: Validity criteria for acute daphnia immobilistaion test according to OECD Guideline 202

	fulfilled	Not fullfilled
Immobilisation of control animals <10%	X	
Control animals not staying at the surface	X	
Concentration of dissolved oxygen in all test vessels >3 mg/l	X	
Concentration of test substance ≥80% of initial concentration during test	X	

Criteria for poorly soluble test substances	X	

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

			Official use only
		1 REFERENCE	
1.1	**Reference**	Bätscher R (2008); DECANOIC ACID: TOXICITY TO *SCENEDESMUS SUBSPICATUS* IN A 72-HOUR ALGAL GROWTH INHIBITION TEST; RCC Ltd, Itingen, Switzerland; Ref nr A7.4.1.3/01D.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	SOPURA N.V.	
1.2.2			
1.2.3	Criteria for data protection	Data on existing a.s. submitted for first time for entry to Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OECD Test Guideline 201 (2006), EU Commission Directive 92/69/EEC, C.3 (1992)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Decanoic acid; as given in section 2	
3.1.1	Lot/Batch number	03108595700	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	99%	
3.1.4	Composition of Product	N.a.	
3.1.5	Further relevant properties	Poorly soluble substance; endogenous substance used by plants and algae used to synthesize C16- and C18 linear fatty acid which will be metabolized to triglycerides of fatty acids and than used to build the double layer of the cell membranes	x
3.1.6	Method of analysis	<u>Analysis of Samples</u> Treatment samples and control samples were thawed at 30° C (water bath) for 1 hour and shaken manually to obtain homogeneous sample solutions. The samples containing 0.1 % phosphoric acid were extracted two times with 50 mL of dichloromethane, each. The combined organic phases were evaporated to dryness using a rotary evaporator (Rotavap) (bath temperature: 40° C, vacuum: 200 mbar, 10 min). The residues were dissolved in 10 mL solvent. It follows a concentration factor of 0.1 (0.04 for the control samples). Aliquots of the final solutions were analyzed by GC. <u>GC Conditions</u>	

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

		Gas chromatograph: AGILENT 6890 Auto sampler: HP 7673A Injection Mode: Pulsed Splitless Injection Volume: 1 µL 4 mm IK Splitless Insert: Glasswool Inlet Purge Time: 0.6 Minute Gas Saver On at 2 Minutes Column: DB WAX (40 m; 0.32 mm inner diameter; 0.50 µm film thickness) Carrier gas: Helium Column Flow: 2.5 ml /Minute Purge Flow: 50 mL/Minute Septum Purge: Constant Temperature program: Injector: 250°C Oven: 60°C, for 1 Minute with 15°C/Minute to 250°C, for 5 Minutes Detector: FID Detector gas (mL/min) H_2 30; Air 400; N_2 30 Retention time: Decanoic acid: approx. 11.3 Minutes Octanoic acid (Internal standard): approx. 9.95 Minutes Octanoic acid was used as internal standard.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Prior to the study, a pre-experiment without GLP was performed to investigate the water solubility and the stability of the test item by analysis. In this pre-experiment, the test item was completely dissolved after stirring for 24 hours. Moreover, the test item was determined to be stable in aqueous media (without algae) over the period of at least 96 hours.	x
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	-	
3.4	**Testing procedure**		
3.4.1	Culture medium		

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

Macro-nutrients:		
	$NaHCO_3$	50.0 mg/L
	$CaCl_2 \times 2\ H_2O$	18.0 mg/L
	NH_4Cl	15.0 mg/L
	$MgSO_4 \times 7\ H_2O$	15.0 mg/L
	$MgCl_2 \times 6\ H_2O$	12.0 mg/L
	KH_2PO_4	1.6 mg/L
Trace elements:		
	$Na_2EDTA \times 2\ H_2O$	100.0 µg/L
	$FeCl_3 \times 6\ H_2O$	80.0 µg/L
	$MnCl_2 \times 4\ H_2O$	415.0 µg/L
	H_3BO_3	185.0 µg/L
	$Na_2MoO_4 \times 2\ H_2O$	7.0 µg/L
	$ZnCl_2$	3.0 µg/L
	$CoCl_2 \times 6\ H_2O$	1.5 µg/L
	$CuCl_2 \times 2\ H_2O$	0.01 µg/L

Calculated water hardness of the test water: 0.24 mmol/L (= 24 mg/L as $CaCO_3$).

3.4.2	Test organisms	See table A7_4_1_3-2
3.4.3	Test system	See table A7_4_1_3-3
3.4.4	Test conditions	See table A7_4_1_3-4
3.4.5	Duration of the test	72 hours
3.4.6	Test parameter	Cell density: the number of cells per mL Growth: the increase of cell density over the test period Yield (y): cell density at the end of the exposure period minus cell density at the start of the exposure period, calculated to express biomass increase during the test Growth rate (µ): the increase of cell density per unit time EγCx: the calculated concentration of test item that results in an x% reduction of yield (y) relative to the control EµCx: the calculated concentration of test item that results in an x% reduction of growth rate (µ) relative to the control NOEC (No Observed Effect Concentration): the highest test concentration at which no significant inhibition of growth is observed relative to the control LOEC (Lowest Observed Effect Concentration): the lowest test concentration at which a significant inhibition of growth is observed relative to the control
3.4.7	Sampling	Small volumes of the test media and the control (1.0 mL) were taken from of all test flasks after 24, 48 and 72 hours of exposure, and were not replaced. The algal cell densities in the samples were determined by counting with an electronic particle counter (Coulter Counter, Model ZM), with at least two measurements per sample. In addition, after 72 hours of exposure, a sample was taken from the control and from a test concentration with reduced algal growth (nominal 32 mg/L). The shape and size of the algal cells were examined microscopically in these samples. This test concentration was chosen

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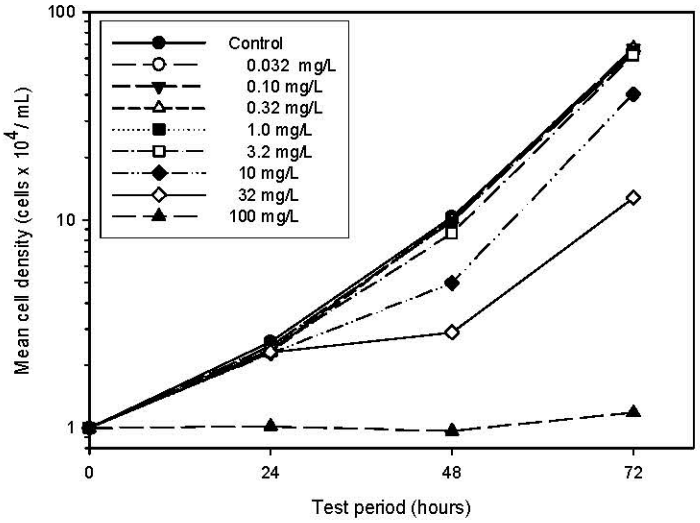
since, at the highest nominal concentration of 100 mg/L, the algal cell density at the end of the test was too low for a reliable microscopic examination.

3.4.8	Monitoring of TS concentration	Yes, interval 24 hours
3.4.9	Statistics	DUNNETT, C.W. (1955): A multiple comparison procedure for comparing several treatments with a control, Journal of the American Statistical Association 50, 1096–1121

4 RESULTS

Limit Test		Not performed
4.1.1	Concentration	-
4.1.2	Number/ percentage of animals showing adverse effects	-
Results test substance		
4.1.3	Initial concentrations of test substance	0, 0.032, 0.10, 0.32, 1.0, 3.2, 10, 32, 100 mg/L
4.1.4	Actual concentrations of test substance	See Table A7_4_1_3-6:
4.1.5	Growth curves	



4.1.6	Concentration / response curve	Based on the measured concentrations of decanoic acid a graphical presentation of the concentration-effect relationship makes no sense.

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4.1.7 Cell concentration data

(see table A7_4_1_3-5)

4.1.8 Effect data (cell multiplication inhibition)

Parameter	Growth rate 0-24 h (mg/L)	Growth rate 24-48 h (mg/L)	Growth rate 48-72 h (mg/L)
EC10	15	0.36	0.14
95% confidence interval	n.d.	0.00 – 0.59	n.d.
EC50	42	0.86	2.0
95% confidence interval	n.d.	0.33 – 1.26	n.d.
EC90	118	2.1	28
95% confidence interval	n.d.	1.4 – 176	n.d.

4.1.9 Other observed effects

The highest dose completely inhibits the growth of the algae. At that dose after 48 hours 90% of the decanoic acid is present. After that time the culture becomes turbid probably because of bacteria growth. At 72 hours only 7.4% decanoic acid is left. This behaviour shows that decanoic acid is easily metabolised or degraded.

All lower doses show a fast disappearance of decanoic acid. At low doses all decanoic acid disappeared after 24 hours. At middle doses it takes longer before all decanoic acid is below the detection limit.

As algae and all plants are autotrophic and build organic matter from sunlight and carbon dioxide, they possess metabolic pathways to synthesize fatty acid and ultimately triglycerides of fatty acid to build the lipid double layers of the cell membranes. These pathways metabolize any intermediate to the next step and if a high level of product is formed in the enzymatic process product inhibition is observed.

Analysing the growth curves and the density of the algae culture, intermediate lowering of the growth in the period of 24 to 48 hours is observed for nominal concentration of 10 and 32 mg/L. This reduction of growth could be explained by the necessity of a metabolic switching in the tri-carboxylic pathway, when the intermediate decanoic acid is consumed and the algae are forced to synthesize the fatty acid again from carbon dioxide instead of the decanoic acid.

Results of controls

In the control, the cell density increased from nominal N = 1 x10^4 cells/mL at the start of the test (0 hours) to N = 65 x 104 cells/mL (mean value) after 72 hours (Table 1). The validity criterion of increase of cell density by at least a factor of 16 within three days was fulfilled. The mean coefficient of variation of the daily growth rates was 32.1% and, thus, lowers than the maximum of 35% given by the OECD test guideline. The coefficient of variation of the average specific growth rates in the control replicates during the test period was 1.1% and, thus,

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below the maximum of 7% indicated by the OECD test guideline. Therefore, all validity criteria given by the test guidelines were fulfilled.

Test with reference substance	Not performed
4.1.10 Concentrations	-
4.1.11 Results	-

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The influence of the test item Decanoic acid on the growth of the freshwater green algal species *Scenedesmus subspicatus* was investigated in a 72-hour static test according to the EU Commission Directive 92/69/EEC, C.3 (1992) and the OECD Test Guideline 201 (2006).

Prior to the study, a pre-experiment without GLP was performed to investigate the water solubility and the stability of the test item by analysis. In this pre-experiment, the test item was completely dissolved after stirring for 24 hours. Moreover, the test item was determined to be stable in aqueous media (without algae) over the period of at least 96 hours.

The following nominal concentrations of Decanoic acid were tested: 0.032, 0.10, 0.32, 1.0, 3.2, 10, 32 and 100 mg/L. Additionally, a control was tested in parallel (test water without test item).

Before the start of the test, the test medium of the highest nominal concentration of 100 mg/L was prepared by dissolving 160.1 mg of test item completely in 1600 mL of test water using ultrasonic treatment (2 x 15 minutes) and intense stirring for 24 hours at room temperature in the dark. The stirring time of 24 hours was chosen based on the results of the pre-experiment mentioned above.

The pH of the test media and the control was adjusted to 7.2-7.6 to lie within the physiological range for the algae. The pH of the test medium of the highest test concentration before adjustment was approximately 6.0 and was adjusted by addition of sodium hydroxide solution (0.1 M).

Adequate volumes of this test medium were diluted with test water to prepare the test media with the lower test item concentrations. Before, the pH of the test water was adjusted from approximately 8.0 to 7.5 by addition of hydrochloric acid solution (0.1 M).

The test media were prepared just before introduction of the algae (i.e., start of the test). The actual concentrations of the test item in the test media were analytically determined.

The selection of the test concentrations was based on the results of two range-finding tests (without GLP). In the first range-finding test, the algal cell densities at the nominal test concentrations of 0.10, 1.0, 10 and 100 mg/L were 87, 52, 33 and 11% of the cell density in the control, respectively, after 72 hours test duration. In the second range-finding test, the algal cell density after 72 hours at the nominal concentrations of 0.010, 0.032, 0.10 and 1.0 mg/L were 102, 104, 128 and 170% of the cell density in the control, respectively. The reason for the inconsistent results may be the fate of the test item (adsorption/uptake or metabolism

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by the algae, degradation etc.). Therefore, a wide concentration range of nominal 0.032 to 100 mg/L was tested using a factor of 3.2 between the test concentrations.

In addition, the concentration of decanoic acid was determined every 24 hours for all nominal concentrations. The analytical method applied was verified and suitable for this test.

5.2 Results and discussion

The influence of the test item decanoic acid on the growth of the freshwater green algal species *Scenedesmus subspicatus* was investigated in a 72-hour static test according to the EU Commission Directive 92/69/EEC, C.3 (1992) and the OECD Test Guideline 201 (2006).

The nominal test concentrations were 0.032, 0.10, 0.32, 1.0, 3.2, 10, 32 and 100 mg/L in parallel with a control. The pH of the test media and the control was adjusted to 7.2-7.6 to lie within the physiological range for the algae.

At the start of the test, the measured concentrations of the test item Decanoic acid in the analyzed test media (nominal test concentrations of 3.2 to 100 mg/L) were between 92 and 95% of the nominal values, demonstrating the correct dosage of the test item. During the test period, a fast decrease of the concentrations of the test item in the test media was determined. The decrease was much faster at the lower test concentrations than at the higher ones.

During the first 24 hours of the test, no statistically significant inhibitory effect was determined at the nominal test item concentrations up to and including 32 mg/L. At the highest nominal test concentration of 100 mg/L, the growth of the algae was completely inhibited. The 24-hour NOEC (highest concentration tested without toxic effects after the test period of 24 hours) was determined to be the nominal concentration of 32 mg/L. The 24-hour LOEC (lowest concentration tested with toxic effects) was determined to be the nominal concentration of 100 mg/L. The mean measured concentrations at the 24-hour NOEC and LOEC were 29 and 94 mg/L, respectively. The calculated 24-hour EC50 for the inhibition of yield and growth rate based on mean measured test item concentrations were 35 and 42 mg/L, respectively (see below).

In the interval 24-48 hours, the sectional growth rate was statistically significantly reduced at nominal test concentrations of 10, 32 and 100 mg/L. In this interval, the concentration of the test item measured at the nominal test concentration of 3.2 mg/L was below the LOQ (limit of quantification) of the analytical method. At the nominal concentrations of 10 and 32 mg/L, the measured concentrations at the beginning of the interval were 5.5 and 28 mg/L, respectively. At the end of the interval, the measured concentrations of the test item were below the LOQ at these test concentrations. At the highest nominal test concentration of 100 mg/L, 93 and 90% of the nominal concentration was measured at the start and the end of the interval, respectively. The results in this second time period contradict the results of the first time period (0-24 hours) at which with the exception of the highest concentration (nominal 100 mg/L, 92.5 mg/mL measured) no effect was found despite measured higher concentrations of decanoic acid.

During the last 24 hours of the test, the sectional growth rate (48-72

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		hours) at the nominal test concentration of 10 mg/L was not inhibited anymore. And also the inhibition of the sectional growth rate at the nominal concentration of 32 mg/L was less than in the interval 24-48 hours. Thus, a recovery of the growth rate of the algae was determined during the last 24 hours of the test. These findings are consistent with the analytical results, which showed that the measured concentrations of the test item at the nominal concentrations of 10 and 32 mg/L were below the LOQ at the start and the end of the last test interval of 24 hours. In addition, 24 hours could be enough to readjust the metabolism of fatty acid synthesis and metabolise the required acid again without using the artificially available decanoic acid. Since the growth inhibition was not constant during the test period, the EC values for yield were calculated for the intervals 0-24, 0-48 and 0-72 hours test duration on the basis of mean measured concentrations in the respective intervals (see 4.1.8). These number may present a realistic worst case as during the first 24 hours with the highest measured concentrations of decanoic acid only the highest concentration 100 mg/mL nominal respectively 94 mg/mL measured was the only concentration causing growth rate inhibition or almost no additional algae cells. For that situation the decanoic acid concentration was stable for the next 24 hours. After that time the culture turned turbid as the result of bacteria growth. Immediately the concentration of decanoic acid declined significantly (7.4 % reminded). At the standard length of the study, the test item had a statistically significant inhibitory effect on the growth (yield and growth rate) of *Scenedesmus subspicatus* after the test period of 72 hours at the nominal test item concentrations of 10 mg/L (= 72-hour LOEC: lowest concentration tested with toxic effects after the test period of 72 hours) and above. The 72-hour NOEC (highest concentration tested without toxic effects) was determined to be the nominal concentration of 3.2 mg/L, since up to and including this test concentration both the mean yield and the mean growth rate of the algae after 72 hours test duration were not statistically significantly lower than in the control.	
5.2.1	NOE_rC	Nominal = 10 mg/L	x
5.2.2	$\mathbf{E_{r50}}$	24 h = 35 mg/L; 48 h = 0.96 mg/L; 72 h = 1.16 mg/L (see discussion)	x
5.2.3	$\mathbf{E_{\mu}X_{50}}$	24 h = 42 mg/L; 48 h = 0.86 mg/L; 72 h = 2.0 mg/L (see discussion)	x
5.3	**Conclusion**	The study was performed completely as required by the new OECD guideline including an improved reporting on the actual concentration of the test item every day and taking the OECD GUIDANCE DOCUMENT ON AQUATIC TOXICITY TESTING OF DIFFICULT SUBSTANCES AND MIXTURES into account. The fact that the test item decanoic acid disappeared fast from the test medium in the presence of algae, while being stable in the test medium for 96 hours, can be explained by the fact that decanoic acid is a endogenic substance of algae which will be directly incorporated into the manifold metabolic pathways to sustain the fast growth of the algae. The results based on measured data do not show the normal inhibitory behaviour expected in this type of study. There is no reason known why	x

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at significant measured concentrations of decanoic acid the substance has no toxic effect on algae and suddenly during the next 24 hours when decanoic acid is significantly depleted from the media, resulting in much lower concentration, starts to exhibit a inhibitory effect. For physiological reasons it could be assumed that the algae during the first 24 hours have used decanoic acid directly for the synthesis of fatty acid. After reduction of the decanoic acid concentration in the medium, the algae were forced to switch the metabolism to a normal situation and synthesis decanoic acid again by themselves. The observed reduction in the growth curves of the algae may be a lag-time effect to readjust to the new medium with low decanoic acid concentration.

Taking the specific properties of decanoic acid into account it is reasonable to conclude, based on nominal concentrations and the shape of the growth curves the following realistic worst-case values:

EC_{50} = 32 mg/L because there is in the period of 24 to 48 hours a reduction of the growth rate and not a complete recovery in the period of 48 to 72 hours.

LOEC = 10 mg/L because there is in the period of 24 to 48 hours a slight reduction of the growth rate and complete recovery in the period of 48 to 72 hours.

NOEC = 3.2 mg/L there is no reduction in the growth curve during the whole period of the test.

5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
Date	[redacted]
Materials and Methods	
Results and discussion	
Conclusion	

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Reliability	[redacted]
Acceptability	
Remarks	

Table A7_4_1_3-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	No
Concentration of vehicle	-
Vehicle control performed	-
Other procedures	Performed according to the latest OECD guideline with analytical determination of the test substance at 0, 24, 48, 72 hours.

Table A7_4_1_3-2: Test organisms

Criteria	Details
Species	*Scenedesmus subspicatus* (now renamed to *Desmodesmus subspicatus)*
Strain	Strain No. 86.81 SAG
Source	Collection of Algal Cultures (SAG, Institute for Plant Physiology, University of Göttingen, D-37073 Göttingen, Germany).
Laboratory culture	Yes
Method of cultivation	Standardized conditions according to the test guidelines by RCC's laboratories
Pretreatment	Exponentially growing pre-culture, which was set up four days prior to the test under the same conditions as in the test.
Initial cell concentration	Nominal 1 x 10^4 cells/ml.

Table A7_4_1_3-3: Test system

Criteria	Details
Volume of culture flasks	50 mL
Culturing apparatus	Temperature controlled water bath at a temperature of 23 °C and continuously illuminated
Light quality	Fluorescent tubes (Philips TLD 36W/840) with a measured light intensity of about 8800 Lux (mean value, range 8210-9240)
Procedure for suspending algae	Stirring
Number of vessels/ concentration	3
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_3-4: Test conditions

Criteria	Details
Test temperature	<table><tr><th></th><th>Temperature (°C)</th></tr><tr><td>Day 0 (Start)</td><td>23</td></tr><tr><td>Day 1</td><td>23</td></tr><tr><td>Day 2</td><td>23</td></tr><tr><td>Day 3 (End)</td><td>23</td></tr></table>
pH	<table><tr><th rowspan="2">Nominal test item concentration (mg/L)</th><th colspan="2">pH values</th></tr><tr><th>Start</th><th>End</th></tr><tr><td>Control</td><td>7.6</td><td>7.7</td></tr><tr><td>0.032</td><td>7.5</td><td>7.8</td></tr><tr><td>0.10</td><td>7.5</td><td>7.8</td></tr><tr><td>0.32</td><td>7.5</td><td>7.8</td></tr><tr><td>1.0</td><td>7.5</td><td>8.0</td></tr><tr><td>3.2</td><td>7.5</td><td>8.0</td></tr><tr><td>10</td><td>7.5</td><td>8.0</td></tr><tr><td>32</td><td>7.4</td><td>7.7</td></tr><tr><td>100</td><td>7.2</td><td>7.2</td></tr></table>
Aeration of dilution water	No
Light intensity	8800 Lux (mean value, range 8210-9240)
Photoperiod	Continuously

Table A7_4_1_3-5: Cell concentration data

Test-Substance Concentration (nominal/[1] [mg/l]	**Cell concentrations (mean values) [x 10^4cells/ml]**							
	measured				**Percent of control**			
	0 h	**24 h**	**48 h**	**72 h**	**0 h**	**24 h**	**48 h**	**72 h**
Control	1.0	2.6	10.3	65.0	-	-	-	-
0.032	1.0	2.5	9.8	63.3	100	96	95	97
0.10	1.0	2.4	10.1	66.9	100	92	98	103
0.32	1.0	2.3	10.1	67.0	100	88	98	103
1.0	1.0	2.5	9.8	65.0	100	96	95	100
3.2	1.0	2.4	8.6	62.0	100	92	83	95
10	1.0	2.3	5.0	40.3	100	88	49	62
32	1.0	2.3	2.9	12.8	100	88	28	20
100	1.0	1.0	1.0	1.2	100	-	-	-
Temperature [°C]	23	23	23	23				
pH	See table	-	-	See table				

[1] specify, if TS concentrations were nominal or measured

Table A7_4_1_3-6: 4.1.4 Actual concentrations of test substance

Nominal concentration of Decanoic acid [mg/L]	Sampling date [day]	Age of sample [hours]	RCC sample code	Decanoic acid measured [mg/L]	[% of nominal]	average [mg/L]	average [% of nominal]
Treatment samples							
3.2	0	0	A11	2.93	91		
	0	0	A12	2.95	92	2.94	92
	1	24	A29	< LOQ	n.a.		
	1	24	A30	< LOQ	n.a.	< LOQ	n.a.
	2	48	A47	< LOQ	n.a.		
	2	48	A48	< LOQ	n.a.	< LOQ	n.a.
	3	72	A65	< LOQ	n.a.		
	3	72	A66	< LOQ	n.a.	< LOQ	n.a.
10	0	0	A13	9.38	94		
	0	0	A14	9.51	95	9.44	94
	1	24	A31	5.31	53		
	1	24	A32	5.68	57	5.50	55
	2	48	A49	< LOQ	n.a.		
	2	48	A50	< LOQ	n.a.	< LOQ	n.a.
	3	72	A67	< LOQ	n.a.		
	3	72	A68	< LOQ	n.a.	< LOQ	n.a.
32	0	0	A15	30.2	94		
	0	0	A16	30.2	94	30.2	94
	1	24	A33	27.8	87		
	1	24	A34	27.9	87	27.8	87
	2	48	A51	< LOQ	n.a.		
	2	48	A52	< LOQ	n.a.	< LOQ	n.a.
	3	72	A69	< LOQ	n.a.		
	3	72	A70	< LOQ	n.a.	< LOQ	n.a.
100	0	0	A17	95.1	95		
	0	0	A18	95.9	96	95.5	95
	1	24	A35	92.4	92		
	1	24	A36	92.9	93	92.6	93
	2	48	A53	89.1	89		
	2	48	A54	90.9	91	90.0	90
	3	72	A71	7.49	7.5		
	3	72	A72	7.33	7.3	7.41	7.4
Biological control samples							
0	0	0	A1	< LOQ	n.a.	n.a.	n.a.
	1	24	A19	< LOQ	n.a.	n.a.	n.a.
	2	48	A37	< LOQ	n.a.	n.a.	n.a.
	3	72	A55	< LOQ	n.a.	n.a.	n.a.
Spiked test water samples							
3.25		0	AZ1	3.35	103		
		0	AZ2	3.36	103	3.36	103
102		0	AZ3	109	108		
		0	AZ4	109	107	109	107
					arithmetic mean :	n.a.	105
Analytical blank							
0		0	AZ0	< LOQ	n.a.	n.a.	n.a

LOQ = 0.218 mg test item /L

n.a. = not applicable

The tabulated values of the samples represent rounded results obtained by calculation using the exact raw data.

3. Tables for Applicant's Summary and Conclusion

3.1 Validity criteria for algal growth inhibition test according to OECD Guideline 201

	fulfilled	Not fullfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	X	
Concentration of test substance ≥80% of initial concentration during test		X

Criteria for poorly soluble test substances	X	

Section A7.4.1.4 Inhibition to microbiological activity (aquatic)

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			Official use only
		1 REFERENCE	
1.1	**Reference**	Seyfried B (2006); DECANOIC ACID: TOXICITY TO ACTIVATED SLUDGE IN A RESPIRATION INHIBITION TEST; RCC Ltd, Itingen Switzerland; RCC Study Number A86545; Ref nr A7.4.1.4/01.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	SOPURA N.V.	
1.2.2			
1.2.3	Criteria for data protection	Data on existing a.s. for first entry to Annex I authorisation	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes; OECD guideline No. 209; EU Directive 88/302/EEC, C.11	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes	x
		3 MATERIALS AND METHODS	
3.1	**Test material**	Decanoic acid as given in section 2	
3.1.1	Lot/Batch number	A0201 703	x
3.1.2	Specification	As given in section 2	
3.1.3	Purity	98.8%	x
3.1.4	Composition of Product	N.a.	
3.1.5	Further relevant properties	Decanoic acid is used as a disinfectant	
3.1.6	Method of analysis	Actual concentration was not determined analytically.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	No stock solution could be prepared due to the limited water solubility of Decanoic acid. The test item was first pulverized in a mortar. Then test item amounts of 5.07, 16.02, 50.00, 160.14 and 500.42 mg were weighed by means of an analytical balance and transferred to the designated test vessels with 284 mL tap water. The test item was mixed into the tap water by ultrasonic treatment for fifteen minutes and intense stirring for 24 hours at room temperature in the dark to dissolve a maximum amount of the test item and/or disperse it as homogeneously as possible. No emulsifiers or solvents were used.	
3.3	**Reference substance**	Yes, 3,5-dichlorophenol	
3.3.1	Method of analysis for reference	Tested at nominal concentrations, completely soluble.	

Section A7.4.1.4 Inhibition to microbiological activity (aquatic)

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	substance		
3.4	**Testing procedure**		
3.4.1	Culture medium	Based on this ratio, an aliquot of washed sludge was suspended in tap water to obtain a concentration equivalent to 3 g dry material per liter. During the holding period of three days prior to use, the sludge was fed daily with 50 mL synthetic wastewater* per litre and was kept at room temperature under continuous aeration until use. Before use, the dry weight of the activated sludge was measured again in the inoculum used for the test. The pH of the activated sludge inoculum was 6.5. * Synthetic wastewater: 16 g peptone 11 g meat extract 3 g urea 0.7 g NaCI 0.4 g CaC12 + 2H20 0.2 g MgSQ . 7H20 2.8 g KaHPQ filled up to a final volume of 1 litre with deionized water.	x
3.4.2	Inoculum / test organism	(see table A7_4_1_4-2)	
3.4.3	Test system	(see table A7_4_1_4-3)	
3.4.4	Test conditions	(see table A7_4_1_4-4) At the start of the test (after the stirring period of 24 hours), synthetic wastewater and activated sludge inoculum (see Sections 2.4 and 2.5.1) were added. The inoculum had a sludge concentration of 2.5 g/L dry weight (corresponding to about 1 g dry material per litre test medium). The sludge was added in time intervals of 15 minutes (an arbitrary but convenient interval) first to a control, secondly to the test solutions of the reference item, thirdly to the test solutions of the test item, and finally to the second control. During the incubation period of 3 hours all test media and the controls were continuously aerated by intense stirring on magnetic stirrers to avoid possible foaming and/or stripping of the test item. The concentration of dissolved oxygen did not drop below 2,5 rng/L during the incubation period. Just before measurement of the respiration rates, the dissolved oxygen concentrations were at least 8.1 mg/L (Table 1). The temperature in the test media, measured in one control, was 21 °C at the start and at the end of the incubation period.	x
3.4.5	Duration of the test	3-hours	
3.4.6	Test parameter	**Respiration inhibition** The inhibitory effect of the test item or of the reference item on the respiration rate (oxygen consumption per minute) is expressed as a percentage of the mean respiration rate of the two controls, measured at	

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		the start and at the end of the test series (vessels A and B). $\left(1-\frac{2R_s}{R_{c1}+R_{c2}}\right)\times 100\% = \text{percent inhibition}$ where R_s = oxygen consumption rate (in mg O_2 L^{-1} $minute^{-1}$) at tested concentration R_{c1} = oxygen consumption rate in the first control vessel R_{c2} = oxygen consumption rate in the second control vessel The 3-hour EC50, EC20 and EC80 of the test item and their 95%-confidence limits could not be calculated because of the absence of a toxic effect. The 3-hour EC50 of the reference item 3,5-dichlorophenol was calculated by Probit Analysis. All test results were based on nominal concentrations of the test and reference item.
3.4.7	Analytical parameter	pH values, oxygen
3.4.8	Sampling	Samples are taken at the end of the 3-hours period. For measurement of the respiration rate, a well-mixed sample of each test medium was poured into a BOD-flask after three hours incubation time, and was not further aerated. Then, the dissolved oxygen concentration was measured with an oxygen electrode (WTW TriOximatic® 300 and an oxygen meter WTW Oxi 539, Wissenschaftlich-Technische Werkstaetten WTW, Weilheim/Germany), and was continuously recorded. During measurement, the samples were continuously stirred on a magnetic stirrer. The oxygen consumption rate (in mg O_2 L^{-1} $minute^{-1}$) was determined from the linear part of the respiration curve in the range of 6.5-2.5 mg O_2/L. In case of very rapid oxygen consumption, the range used was below the limits indicated above, but always within the linear part of the respiration curve. In case of low oxygen consumption the rate was determined over a period of at least ten minutes.
3.4.9	Monitoring of TS concentration	No
3.4.10	Controls	Tap water, synthetic waste water and inoculum
3.4.11	Statistics	Probit Analysis:
		4 RESULTS
Preliminary test		Not performed
4.1.1	Concentration	-
4.1.2	Effect data	-

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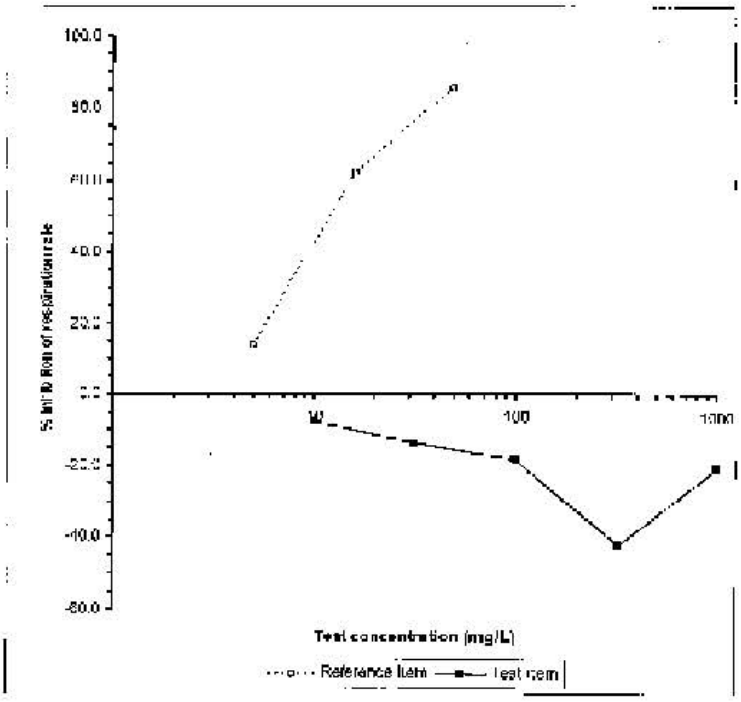
Results test substance

4.1.3	Initial concentrations of test substance	10, 32, 100, 320, 1000 mg/L
4.1.4	Actual concentrations of test substance	Not measured, suspension
4.1.5	Growth curves	

Figure 1. Influence of Decanoic acid and 3,5-dichlorophenol on the respiration rate of activated sludge



4.1.6	Cell concentration data	Not reported

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4.1.7 Concentration/ response curve

Table 1: Influence of Decanoic acid and 3,5-dichlorophenol on the oxygen consumption of activated sludge

Vessel no.	Nominal concentration of test chemical (mg/L)	Oxygen consumption rate (mg O_2/L·min)	Inhibition (%)	pH values start*	pH values end*	Oxygen concentration (mg O_2/L) start*	Oxygen concentration (mg O_2/L) end*
Control							
A	0	1.576		7.3	8.0	8.0	8.6
B	0	1.470		7.4	8.1	8.3	8.4
Mean		1.523					
Deviation (%)		6.7					
Test item							
4	10	1.644	-7.9	7.2	8.0	7.8	8.4
5	32	1.735	-13.9	7.1	7.9	7.5	8.1
6	100	1.803	-18.2	7.3	8.0	7.4	8.6
7	320	2.157	-42.3	7.2	7.9	7.6	8.1
8	1000	1.840	-20.8	6.8	7.8	6.3	8.7
Reference item							
1	5	1.314	13.7	7.2	8.1	8.1	8.4
2	16	0.575	62.2	7.3	8.1	8.4	8.1
3	50	0.226	85.2	7.3	8.1	8.2	8.6

* Start and end of the 3-hour incubation period
- % inhibition increases oxygen consumption rate relative to control

4.1.8 Effect data

No inhibition observed.

4.1.9 Other observed effects

None

Results of controls

Test with reference substance

Performed

4.1.10 Concentrations

5, 16, 50 mg/L

4.1.11 Results

The 3-hour EC50 of the reference item 3,5-dichlorophenol (positive control) was calculated to be 14 mg/L (the 95% confidence limits were not calculable). The 3-hour EC50 is within the guideline-recommended range of 5-30 mg/L, confirming suitability of the activated sludge used.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The inhibitory effect of the test item Decanoic acid on the respiration rate of aerobic wastewater microorganisms of activated sludge was investigated in a 3-hour respiration inhibition test according to the EU Commission Directive 88/302/EEC, Part C.11, and the OECD Guideline for Testing of Chemicals, No. 209.

The following nominal concentrations of decanoic acid were tested: 10, 32, 100, 320 and 1000 mg/L.

In addition, two controls and three different concentrations of the reference item 3,5-dichlorophenol (5, 16 and 50 mg/L) were tested in parallel. The test is regarded as valid since the oxygen consumption rates of the two controls at the start and the end of the test differed only by 7% and the 3-hour EC50 of the positive control 3,5-dichlorophenol was 14 mg/L. Both values are within the guideline-recommended range (i. e., maximum variation of 15% between the two controls and an EC50

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		of 5-30 rng/L for 3,5-dichlorophenol).
5.2	**Results and discussion**	Down to the lowest test concentration of 10 mg/L, at least part of the test item was not dissolved in the test media (assessed before the addition of activated sludge and synthetic wastewater). However, it can be assumed that the test item was dissolved during the 3-hour incubation period since the test item was ready biodegradable (10% degradation within the first 24 hours and about 60% degradation after five days of incubation; RCC Study No. A86567 - Decanoic acid: Ready biodegradability in a manometric respirometry test; see A7.1.1.2.1). Up to and including the highest test concentration of nominal 1000 mg/L, the test item had no inhibitory effect (<15% inhibition) on the respiration rate of activated sludge after the incubation period of three hours, Moreover, the respirations rates measured in the presence of Decanoic acid were higher than those in the controls. Up to and including the test item concentration of nominal 320 mg/L, respiration rates were 8% to 42% higher than in the controls. At nominal 1000 mg/L, the respiration rate was stimulated by 21% compared to the controls. The increased respiration rates most probably can be attributed to rapid degradation of the readily biodegradable test item (see above). In conclusion, the 3-hour NOEC (EC15) of decanoic acid to activated sludge microorganisms was at least 1000 mg/L, This value might even be higher but concentrations above 1000 mg/L were not tested. The 3-hour EC20, EC50, and EC80 could not be calculated but were clearly higher than 1000 mg/L
5.2.1	EC_{20}	> 1000 mg/L
5.2.2	EC_{50}	> 1000 mg/L
5.2.3	EC_{80}	> 1000 mg/L
5.3	**Conclusion**	The test item Decanoic acid has no inhibitory effect on the respiration rate of activated sludge after the incubation period of three hours up to and including the test item concentration of 1000 mg/L.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities
EVALUATION BY RAPPORTEUR MEMBER STATE

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Date	[redacted]
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_4_1_4-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes
Vehicle	No
Concentration of vehicle	-
Vehicle control performed	No
Other procedures	None

Table A7_4_1_4-2: Inoculum / Test organism

Criteria	Details
5.3.3 Nature	Activated sludge
5.3.4 Species	-
5.3.5 Strain	-
5.3.6 Source	Sewage treatment plant treating predominantly domestic sewage
5.3.7 Sampling site	ARA Ergolz II, Füllinsdorf, Switzerland
5.3.8 Laboratory culture	No
5.3.9 Method of cultivation	-
5.3.10 Preparation of inoculum for exposure	The sludge was washed twice with tap water by centrifugation and the supernatant liquid phase was decanted. A homogenized aliquot of the final sludge suspension was weighed, thereafter dried and the ratio of wet to dry weight was calculated.
5.3.11 Pretreatment	Preculture An aliquot of washed sludge was suspended in tap water to obtain a concentration equivalent to 3 g dry material per litre. During the holding period of three days prior to use, the sludge was fed daily with 50 mL synthetic wastewater per liter and was kept at room temperature under continuous aeration until use.
5.3.12 Initial cell concentration	Concentration equivalent to 3 g dry material per litre

Table A7_4_1_4-3: Test system

Criteria	Details
5.3.13 Culturing apparatus	2000 mL glass beaker
5.3.14 Number of culture flasks/concentration	1
5.3.15 Aeration device	Intense stirring on magnetic stirrers
5.3.16 Measuring equipment	pH-electrode, O_2-electrode
5.3.17 Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_4-4: Test conditions

Criteria	**Details**
5.3.18 Test temperature	*Give measurements conducted during test*
5.3.19 pH	*Give measurements conducted at start and end of test*
5.3.20 Aeration of dilution water	Yes/No *(If yes, specify: e.g. air-flow)*
5.3.21 Suspended solids concentration	

Section A7.4.2 Annex Point IIA7.5	Bioconcentration in aquatic organisms	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] Limited exposure [X]	Technically not feasible [ ] Scientifically unjustified [X] Other justification [ ]	
Detailed justification:	[redacted]	X X
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
Date Evaluation of applicant's justification Conclusion	[redacted]	

Section A7.4.2 Annex Point IIA7.5	Bioconcentration in aquatic organisms
Remarks	[redacted]

Section A7.5.1.1 Annex Point IIA7.4	Inhibition to microbial activity (terrestrial)	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] Limited exposure [ ]	Technically not feasible [ ] Scientifically unjustified [X] Other justification [X]	
Detailed justification:	[redacted]	X
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
Date Evaluation of applicant's justification Conclusion Remarks	[redacted]	

Section A7.5.1.2 Annex Point IIIA XIII 3.2	Earthworm, acute toxicity test	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] Limited exposure [X]	Technically not feasible [ ] Scientifically unjustified [X] Other justification [ ]	
Detailed justification:	[redacted]	X
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
Date Evaluation of applicant's justification Conclusion Remarks	[redacted]	