

Section A7.4.1.1/01 Acute toxicity to fish**Annex Point IIA7.1***Pimephales promelas*Official
use only**1 REFERENCE**

1.1 Reference [REDACTED] (1984) Acute toxicities of organic chemicals to fathead minnows (*Pimephales promelas*), [REDACTED]
[REDACTED]

1.2 Data protection No

1.2.1 Data owner

1.2.2 Criteria for data protection No data protection claimed.

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study No. [REDACTED]
[REDACTED]

2.2 GLP [REDACTED]

2.3 Deviations Not applicable

3 MATERIALS AND METHODS

3.1 Test material Propan-2-ol

3.1.1 Lot/Batch number -

3.1.2 Specification 2-Propanol

3.1.3 Purity Reagent grade (one test) and 99.8 % (two tests)

3.1.4 Composition of Product Not applicable

3.1.5 Further relevant properties The test was conducted under flow-through conditions.

3.1.6 Method of analysis GLC (Gas Liquid Chromatography)

3.2 Preparation of TS solution for poorly soluble or volatile test substances Propan-2-ol is indefinitely miscible with water (cf. Doc IIIA3.5). Based on the measured Henry's Law Constant propan-2-ol is moderately volatile from aqueous solution (cf. Doc IIIA3.2.1).

Continuously the test compound was added undiluted to the exposure tanks. The test was conducted under flow-through conditions.

3.3 Reference substance No data.

3.3.1 Method of analysis for reference substance -

3.4 Testing procedure

3.4.1 Dilution water

Criteria	Details
Source	Lake Superior
Alkalinity	39.5 - 45.9 mg/L (CaCO ₃)
Hardness	44 - 52.5 mg/L (CaCO ₃)

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3.4.2 Test organisms

pH	7.09 - 7.87
Oxygen content	6.6 - 6.7 mg/L
Conductance	No data
Holding water different from dilution water	No data.
Criteria	Details
Species/strain	<i>Pimephales promelas</i> (fathead minnow)
Source	US EPA Environmental Research Laboratory-Duluth
Wild caught	No
Age/size	29 - 31 days/ mean length: 17.4 - 20.6 mm; mean weight: 0.08 - 0.11 g
Kind of food	Before testing: <i>Artemia</i> sp.; during the test fish were not fed
Amount of food	No data.
Feeding frequency	No data.
Pretreatment	Fish were held at 25 °C in flowing water with 16 h light/8 h dark (no further information).
Feeding of animals during test	No.

3.4.3 Test system

Criteria	Details
Test type	Flow-through
Renewal of test solution	flow-rates: 23.6, 36.0, and 72.05 L/d
Volume of test vessels	1, 5.5, and 6.3 L
Volume/animal	0.252, 0.22, and 0.05 L/animal
Number of animals/vessel	20 - 25
Number of vessels/concentration	5 - 10 (some concentrations were tested in duplicate)
Test performed in closed vessels due to significant volatility of TS	No

3.4.4 Test conditions

Criteria	Details
Test temperature	24.4- 24.6 °C
Dissolved oxygen	6.6 - 6.7 mg/L
pH	7.09 - 7.87
Adjustment of pH	No

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Aeration of dilution water	No data
Intensity of irradiation	No data
Photoperiod	16 h light daily

- 3.4.5 Duration of the test 96 h
- 3.4.6 Test parameter Mortality and behavior
- 3.4.7 Sampling Test exposure chambers were sampled at 0 and 96 h and one of the duplicate exposure chambers at 24, 48, and 72 h. All samples were analysed immediately or adequately preserved for later analysis (no further data given).
- 3.4.8 Monitoring of TS concentration Yes. Test exposure chambers were sampled at 0 and 96 h and one of the duplicate exposure chambers at 24, 48, and 72 h. All samples were analysed immediately or adequately preserved for later analysis.
- 3.4.9 Statistics Estimation of LC₅₀ and EC₅₀ was made by using the "trimmed Spearman-Kärber" method (Hamilton et al. (1977) Environ Sci Technol 11, 714-719 and Correction 12, 417 (1978).

4 RESULTS

4.1 Limit Test No data

- 4.1.1 Concentration -
- 4.1.2 Number/
percentage of
animals showing
adverse effects -
- 4.1.3 Nature of adverse effects -

4.2 Results test substance

- 4.2.1 Initial concentrations of test substance
Test 1: 3800, 6300, 10500, 17600, 29300 mg/L
Test 2: 2210, 3670, 6120, 10200, and 17000 mg/L
Test 3: 3080, 3760, 5500, 8280, and 13000 mg/L
(all values cited here refer to nominal concentrations)

- 4.2.2 Actual concentrations of test substance Actual concentration determined analytically (in mg/L)

Test 1

nominal	3800	6300	10500	17600	29300
effectiv	2620/ 2810	4790/ 4790	7570/ 7720	13400/ 13700	21700/ 22500/

Test 2

nominal	2210	3670	6120	10200	17000
effectiv	1260/ 1300	2500/ 2590	4320/ 4580	7720/ 7760	14200/ 14400

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Test 3

nominal	3080	3760	5500	8280	13000
effectiv	3090	3760	5500	8280	1300

4.2.3 Effect data
(Mortality)

Test 1

Test substance Concentration (measured) [mg/L]	Mortality							
	Number				Percentage			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
<250 (control)	0	0	0	0	0	0	0	0
2620-2810	0	0	0	0	0	0	0	0
4790	0	0	0	0	0	0	0	0
7570-7720	0	1	4	5	0	2	8	10
13400-13700	50	50	50	50	100	100	100	100
21700-22500	50	50	50	50	100	100	100	100
Temp. [°C]				24.4				
pH				7.79				
Oxygen [mg/l]				6.6				

Test 2

Test substance Concentration (measured) [mg/L]	Mortality							
	Number				Percentage			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
0 (control)	0	0	0	0	0	0	0	0
1260-1300	0	0	0	0	0	0	0	0
2500-2590	0	0	0	0	0	0	0	0
4320-4580	0	0	0	0	0	0	0	0
7720-7760	0	1	1	1	0	2	2	2
14200-14400	50	50	50	50	100	100	100	100
Temp. [°C]				24.6				
pH				7.09				
Oxygen [mg/l]				6.7				

Test 3

Test substance Concentration (measured) [mg/L]	Mortality							
	Number				Percentage			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
<250 (control)	0	0	0	0	0	0	0	0
3090	2	2	2	2	10	10	10	10
3760	1	1	1	1	5	5	5	5
5500	5	5	5	5	25	25	25	25
8280	16	16	16	16	80	80	80	80
13000	20	20	20	20	100	100	100	100
Temp. [°C]				24.6				
pH				7.87				
Oxygen [mg/l]				6.7				

96 h LC₅₀ values obtained in the three tests

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	48 h [mg/L] ¹	95 % c.l.	96 h [mg/L] ¹	95 % c.l.
LC ₀	-	-	-	-
LC ₅₀			6550 (m) 9640 (m) 10400 (m)	5770-7450 9230-10000 10200-10600
LC ₁₀₀	-	-	-	-

¹ measured (m) concentrations

4.2.4 Concentration / response curve See effect data for information regarding concentration/ response relationship.

4.2.5 Other effects In the test 96 h EC₅₀ values were determined also (96 h EC₅₀ = 6120 - 9540 mg/L). Affected fish lost equilibrium prior to death.

4.3 Results of controls

4.3.1 Number/ percentage of animals showing adverse effects No mortalities occurred in the controls (test substance omitted) in test 1 and 2. In test 3 investigating the behavior one affected fish was observed (corresponding to 5% of control fish) 3 hours after initiating the test. No adverse effects or mortalities occurred in test 3 examining lethality.

4.3.2 Nature of adverse effects In test 3 one affected fish was observed in the controls (test substance omitted). The affected fish lost equilibrium prior to death. No other adverse effects were seen.

4.4 Test with reference substance No data.

4.4.1 Concentrations -

4.4.2 Results -

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods The test was conducted under flow-through conditions and was similar to OECD Guideline 203 'Fish, Acute toxicity test'.

At total 3 different tests were conducted and using 5 concentrations each and one control. 20 - 25 fish were used per concentration. Partly, the tests were performed in duplicate (before 1 December 1981). Lake Superior water was used as dilution water. The actual concentration of test substance was determined analytically.

5.2 Results and discussion The substance shows a very low acute toxicity towards *Pimephales promelas* (96 h LC₅₀ = 6550-10400 mg/L; 96 h EC₅₀ = 6120-9540 mg/L).

	fulfilled	Not fulfilled
Mortality of control animals <10%	yes	
Concentration of dissolved oxygen in all test vessels > 60% saturation	yes	
Concentration of test substance ≥80% of initial concentration during test	yes	

The acute toxicity towards *Pimephales promelas* was determined in a study conducted similar to OECD Guideline 203. The test was performed under flow-through conditions and the actual substance

x

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concentration was measured periodically. The study is very well documented and meets the validity criteria.

The test was performed under flow-through conditions and the actual substance concentration was measured periodically. Therefore, the volatility (**cf. Doc IIIA3.2.1**) of the test substance does not play an important role in the present study.

5.2.1	LC ₀	-	
5.2.2	LC ₅₀	96 h LC ₅₀ : 6550 mg/L; 9640 mg/L; 10400 mg/L (effective)	x
5.2.3	LC ₁₀₀	-	
5.3	Conclusion	[REDACTED]	
5.3.1	Other Conclusions	[REDACTED]	
5.3.2	Reliability	[REDACTED]	
5.3.3	Deficiencies	[REDACTED]	

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	2008/06/30
Materials and Methods	[REDACTED]
Results and discussion	[REDACTED]
Conclusion	[REDACTED]
Reliability	[REDACTED]
Acceptability	[REDACTED]
Remarks	[REDACTED]

	COMMENTS FROM ...
Date	<i>Give date of comments submitted</i>
Materials and Methods	<i>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</i>
Results and discussion	<i>Discuss if deviating from view of rapporteur member state</i>
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>
Reliability	<i>Discuss if deviating from view of rapporteur member state</i>
Acceptability	<i>Discuss if deviating from view of rapporteur member state</i>
Remarks	

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Annex Point IIA7.1 *Pimephales promelas*

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1 REFERENCE

1.1 Reference [REDACTED] (1983) Estimating the acute toxicity of narcotic industrial chemicals to fathead minnows. [REDACTED]
[REDACTED]

1.2 Data protection No.
1.2.1 Data owner -
1.2.2 Criteria for data protection No data protection claimed

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study No. [REDACTED]
[REDACTED]
2.2 GLP [REDACTED]
2.3 Deviations No information

3 MATERIALS AND METHODS

3.1 Test material Propan-2-ol
3.1.1 Lot/Batch number -
3.1.2 Specification 2-Propanol
3.1.3 Purity Purity not stated
3.1.4 Composition of Product Not applicable.
3.1.5 Further relevant properties -
3.1.6 Method of analysis UV-spectroscopy or GC/FID
3.2 Preparation of TS solution for poorly soluble or volatile test substances Propan-2-ol is indefinitely miscible with water (cf. Doc III A3.5). Based on the measured Henry's Law Constant propan-2-ol is moderately volatile from aqueous solution (cf. Doc III A3.2.1). The test was conducted under flow-through conditions.
3.3 Reference substance No information
3.3.1 Method of analysis for reference substance -

3.4 Testing procedure

3.4.1 Dilution water	Criteria	Details
	Source	Lake Superior
	Alkalinity	42.2 mg/L as CaCO ₃
	Hardness	56.3 mg/L as CaCO ₃

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3.4.2 Test organisms

pH	7.5
Oxygen content	> 60% of saturation
Conductance	No data
Holding water different from dilution water	No
Criteria	Details
Species/strain	<i>Pimephales promelas</i>
Source	Environmental Research Laboratory-Duluth
Wild caught	No
Age/size	30 days old/ weight 0.12 g
Kind of food	No data
Amount of food	No data
Feeding frequency	No data
Pretreatment	No data
Feeding of animals during test	No

3.4.3 Test system

Criteria	Details
Test type	Flow-through
Renewal of test solution	No information
Volume of test vessels	No information
Volume/animal	No information
Number of animals/vessel	25
Number of vessels/concentration	2
Test performed in closed vessels due to significant volatility of TS	No. However, the test was conducted under flow-through conditions.

3.4.4 Test conditions

Criteria	Details
Test temperature	25 ± 1 °C
Dissolved oxygen	> 60% of saturation
pH	7.5 (pH of dilution water; pH during the test not stated)
Adjustment of pH	No data
Aeration of dilution water	No information
Intensity of irradiation	No information
Photoperiod	No information

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- 3.4.5 Duration of the test 96 h
- 3.4.6 Test parameter Mortality
- 3.4.7 Sampling Dead fish were recorded after 1, 3, 6, 12, 24, 48, 72, and 96 h
- 3.4.8 Monitoring of TS concentration Yes. Concentrations were measured throughout the test.
- 3.4.9 Statistics The LC₅₀ was calculated using the trimmed Spearman-Kärber method

4 RESULTS

4.1 Limit Test No information

- 4.1.1 Concentration -
- 4.1.2 Number/percentage of animals showing adverse effects -
- 4.1.3 Nature of adverse effects -

4.2 Results test substance

- 4.2.1 Initial concentrations of test substance Five concentrations and one control were tested in duplicate (no further information). Test was performed twice.
- 4.2.2 Actual concentrations of test substance Concentrations measured throughout the test (no further information provided).
- 4.2.3 Effect data (Mortality) 96 h LC₅₀ values based on measured concentrations

	48 h [mg/L]	95 % c.l.	96 h [mg/L]	95 % c.l.
LC ₀				
LC ₅₀			9640 and 10000 (measured)	
LC ₁₀₀				

- 4.2.4 Concentration / response curve No information.
- 4.2.5 Other effects No information.
- 4.3 Results of controls** No information.
- 4.3.1 Number/percentage of animals showing adverse effects -
- 4.3.2 Nature of adverse effects -

4.4 Test with reference No information

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substance

4.4.1 Concentrations -

4.4.2 Results -

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The study was conducted according to US EPA (1975) Toxicity tests with aquatic organisms. Twenty-five fish (*Pimephales promelas*) per concentration were exposed to 2-propanol. Six concentrations (incl. control) were tested under flow-through conditions; the tests were conducted in duplicate. Lake Superior water was used as dilution water in all tests. The concentration of the test substance was monitored continuously during the test.

5.2 Results and discussion

	fulfilled	Not fulfilled
Mortality of control animals <10%	No data	
Concentration of dissolved oxygen in all test vessels > 60% saturation	Yes	
Concentration of test substance ≥80% of initial concentration during test	-*	

*concentration of test substance was monitored analytically throughout the test

The study was conducted according to national standard method under flow-through conditions. Some details (e.g. mortality of control animals, concentrations tested) are not reported. However, deviations from the standard procedure and/or special observations during the test are not reported. The concentration of the test substance was measured throughout the test and so the test results refer to effective concentrations. Based on the informations provided it can be assumed that all validity criteria were fulfilled.

Propan-2-ol showed a very low acute toxicity towards *Pimephales promelas* (96 h LC₅₀=9640 and 10000 mg/L effective).

No information on dose-response relationship is available.

The test was performed under flow-through conditions and the actual substance concentration was measured periodically. Therefore the volatility (cf. Doc III A3.2.1) of the test substance does not play an important role in the present study.

5.2.1 LC₀

5.2.2 LC₅₀

96 h LC₅₀ = 9640 and 10000 mg/L (effective)

5.2.3 LC₁₀₀

5.3 Conclusion

[Redacted]

5.3.1 Other Conclusions

5.3.2 Reliability

[Redacted]

5.3.3 Deficiencies

[Redacted]

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Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	2008/06/30
Materials and Methods	[REDACTED]
Results and discussion	[REDACTED]
Conclusion	[REDACTED]
Reliability	[REDACTED]
Acceptability	[REDACTED]
Remarks	[REDACTED]
COMMENTS FROM ...	
Date	<i>Give date of comments submitted</i>
Materials and Methods	<i>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</i>
Results and discussion	<i>Discuss if deviating from view of rapporteur member state</i>
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>
Reliability	<i>Discuss if deviating from view of rapporteur member state</i>
Acceptability	<i>Discuss if deviating from view of rapporteur member state</i>
Remarks	

██████████ A7.4.1.1/03 Acute toxicity to fish
Annex Point IIA7.1 *Oryzias latipes*

Official
use only

1 REFERENCE

- 1.1 Reference** ██████████ (1998) Final Report, Acute Toxicity Test on *Oryzias latipes* to 2-Propanol. ██████████
██████████
- ██████████ 2007 Chemical Risk Information Platform (CHRIP) Total Search System for Chemical Substances: 2-Propanol; ██████████
██████████
- 1.2 Data protection** No.
- 1.2.1 Data owner -
- 1.2.2 Criteria for data protection No data protection claimed

2 GUIDELINES AND QUALITY ASSURANCE

- 2.1 Guideline study** Yes. ██████████
- 2.2 GLP** ██████████
- 2.3 Deviations** None

3 MATERIALS AND METHODS

- 3.1 Test material** Propan-2-ol
- 3.1.1 Lot/Batch number ██████████
- 3.1.2 Specification 2-Propanol
- 3.1.3 Purity ██████████
- 3.1.4 Composition of Product ██████████
██████████
- 3.1.5 Further relevant properties ██████████
██████████
██████████
██████████
██████████
██████████
██████████
██████████
- 3.1.6 Method of analysis ██████████
- 3.2 Preparation of TS solution for poorly soluble or volatile test substances** ██████████
██████████
- 3.3 Reference substance** Yes: copper sulfate pentahydrate
- 3.3.1 Method of analysis for reference No data

[REDACTED] A7.4.1.1/03 Acute toxicity to fish
Annex Point IIA7.1 *Oryzias latipes*

substance

3.4 Testing procedure

3.4.1 Dilution water

[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]

3.4.2 Test organisms

[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]

3.4.3 Test system

[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]

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	██████████	
	██████████	██████████
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

4 RESULTS

4.1 Limit Test

4.1.1 Concentration

4.1.2 Number/
percentage of
animals showing
adverse effects

4.1.3 Nature of adverse effects Not applicable

4.2 Results test substance

4.2.1 Initial concentrations of test substance Not applicable

4.2.2 Actual Not applicable

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- concentrations of test substance
- 4.2.3 Effect data (Mortality) Not applicable
- 4.2.4 Concentration / response curve Not applicable
- 4.2.5 Other effects Not applicable

4.3 Results of controls

- 4.3.1 Number/ percentage of animals showing adverse effects Not applicable
- 4.3.2 Nature of adverse effects Not applicable

4.4 Test with reference substance
Copper sulfate pentahydrate

- 4.4.1 Concentrations No data
- 4.4.2 Results 96 h LC₅₀ = 0.930 mg/L

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

5.2 Results and discussion

5.2.1 LC₀

5.2.2 LC₅₀

5.2.3 LC₁₀₀

5.3 Conclusion

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Annex Point IIA7.2

Daphnia magna STRAUS

Official
use only

1 REFERENCE

- 1.1 Reference** [REDACTED] (1998) Final Report, Acute Immobilisation Test of 2-Propanol on *Daphnia Magna*. [REDACTED]
[REDACTED]
[REDACTED] 2007 Chemical Risk Information Platform (CHRIP) Total Search System for Chemical Substances: 2-Propanol; [REDACTED]
[REDACTED]

1.2 Data protection

- 1.2.1 Data owner No
1.2.2 Criteria for data protection Not applicable

2 GUIDELINES AND QUALITY ASSURANCE

- 2.1 Guideline study** Yes. [REDACTED]
[REDACTED]
2.2 GLP [REDACTED]
2.3 Deviations None

3 MATERIALS AND METHODS

- 3.1 Test material** Propan-2-ol
3.1.1 Lot/Batch number [REDACTED]
3.1.2 Specification [REDACTED]
3.1.3 Purity [REDACTED]
3.1.4 Composition of Product [REDACTED]
3.1.5 Further relevant properties [REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
3.1.6 Method of analysis [REDACTED]
3.2 Preparation of TS solution for poorly soluble or volatile test substances [REDACTED]
3.3 Reference substance Potassium dichromate

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Annex Point IIA7.2

Daphnia magna STRAUS

3.4.3 Test system

[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]

3.4.4 Test conditions

[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]

3.4.5 Duration of the test

[REDACTED]

3.4.6 Test parameter

[REDACTED]

3.4.7 Sampling

[REDACTED]

3.4.8 Monitoring of TS concentration

[REDACTED]

3.4.9 Statistics

[REDACTED]

4 RESULTS

4.1 Limit Test

[REDACTED]

4.1.1 Concentration

[REDACTED]

4.1.2 Number/
percentage of
animals showing
adverse effects

[REDACTED]

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4.1.3	Nature of adverse effects	Not applicable
4.2	Results test substance	Not applicable
4.2.1	Initial concentrations of test substance (mg/L)	Not applicable
4.2.2	Actual concentrations of test substance	Not applicable
4.2.3	Effect data (Immobilisation)	Not applicable
4.2.4	Concentration / response curve	Not applicable
4.2.5	Other effects	No data
4.3	Results of controls	No immobilisation during the exposure period was observed.
4.4	Test with reference substance	Potassium dichromate
4.4.1	Concentrations	No data
4.4.2	Results	48 h EC ₅₀ = 0.141 mg/L

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

[Redacted]

5.2 Results and discussion

[Redacted]

[Redacted]	[Redacted]	[Redacted]
[Redacted]	[Redacted]	[Redacted]
[Redacted]	[Redacted]	[Redacted]
[Redacted]	[Redacted]	[Redacted]
[Redacted]	[Redacted]	[Redacted]

[Redacted]

Section A7.4.1.2/01 Acute toxicity to invertebrates

Annex Point IIA7.2

Daphnia magna STRAUS

5.2.1 EC₀

[REDACTED]

5.2.2 EC₅₀

[REDACTED]

5.2.3 EC₁₀₀

[REDACTED]

5.3 Conclusion

[REDACTED]

5.3.1 Reliability

[REDACTED]

5.3.2 Deficiencies

[REDACTED]

Evaluation by Competent Authorities

Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

EVALUATION BY RAPPORTEUR MEMBER STATE

Date 2014/02/12

Materials and Methods

[REDACTED]

Results and discussion

[REDACTED]

Conclusion

[REDACTED]

Reliability

[REDACTED]

Acceptability

[REDACTED]

Remarks

[REDACTED]

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

Section A7.4.1.2/02 Acute toxicity to invertebrates

Annex Point IIA7.2

Daphnia magna STRAUSOfficial
use only**1 REFERENCE**

- 1.1 Reference** Bringmann G, Kuehn R (1977) Befunde der Schadwirkung wasser-gefährdender Stoffe gegen *Daphnia magna*. Z Wasser Abwasser-Forsch 10, 161-166 (published)
- Bringmann G, Kuehn R (1982) Results of toxic action of water pollutants on *Daphnia magna* Straus tested by improved standardized procedure. Z Wasser Abwasser-Forsch 15, 1-6 (published)

- 1.2 Data protection** No
- 1.2.1 Data owner -
- 1.2.2 Criteria for data protection No data protection claimed

2 GUIDELINES AND QUALITY ASSURANCE

- 2.1 Guideline study** No. Study conducted according to national standard method (German DIN 38412 part 11: *Daphnia* short-term test, 1982, draft)

2.2 GLP

■

- 2.3 Deviations** No

3 MATERIALS AND METHODS

- 3.1 Test material** Propan-2-ol
- 3.1.1 Lot/Batch number -
- 3.1.2 Specification Propanol-(2)
- 3.1.3 Purity No data
- 3.1.4 Composition of Product Not applicable
- 3.1.5 Further relevant properties Although the test substance is moderately volatile the test vessels were only loosely covered with filter paper.
- 3.1.6 Method of analysis No analytical monitoring performed.
- 3.2 Preparation of TS solution for poorly soluble or volatile test substances** Propan-2-ol is indefinitely miscible with water (cf. Doc III A3.5). Based on the measured Henry's Law Constant propan-2-ol is moderately volatile from aqueous solution (cf. Doc III A3.2.1). The test vessels were only loosely covered with filter paper. Based on the short exposure period no significant losses due to volatilisation are assumed.
- 3.3 Reference substance** No data
- 3.3.1 Method of analysis for reference substance -

3.4 Testing procedure

- 3.4.1 Dilution water

Criteria	Details
Source	Artificial fresh water prepared according to DIN 38412 part 11

Section A7.4.1.2/02

Acute toxicity to invertebrates

Annex Point IIA7.2

Daphnia magna STRAUS

3.4.2 Test organisms

Alkalinity	-
Hardness	-
pH	8.0
Ca / Mg ratio	2.5 mmol/L
Na / K ratio	10:1
Oxygen content	maximum
Conductance	-
Holding water different from dilution water	Yes. Holding water: distilled water; Dilution water: prepared acc. to DIN 38412 part 11
Criteria	Details
Species	<i>Daphnia magna</i>
Strain	IRCHA
Source	Laboratory culture
Age	≤24 h
Breeding method	Continuous culture: daily females were transferred to freshly prepared culture glasses; young animals were separated by sieving (DIN filter: 0.315 mm); culture glasses were covered with watch glasses on white tables; cultures were feed daily with dry food (Mikrozell); on Monday and Friday the tape water of all cultures was renewed and also the culture vessels on Friday; for breeding tape water was used (hardness: 16° d.H., pH 7.6 - 7.7); daphnids were cultured at 20 °C; light intensity: E = 2.5 W/m ² ; photoperiod: 9 h light and 15 h dark.
Kind of food	Dry food 'Mikrozell'
Amount of food	30 g/L tap water were suspended; 10 mL of the suspension were added to the culture glasses
Feeding frequency	Daily
Pretreatment	No
Feeding of animals during test	No
Criteria	Details
Renewal of test solution	No
Volume of test vessels	50 mL
Volume/animal	2 mL/animal

3.4.3 Test system

Section A7.4.1.2/02

Acute toxicity to invertebrates

Annex Point IIA7.2

Daphnia magna STRAUS

	Number of animals/vessel	10																
	Number of vessels/ concentration	2																
	Test performed in closed vessels due to significant volatility of TS	No. The test vessels were only loosely covered with filter paper.																
3.4.4	Test conditions	<table border="1"> <thead> <tr> <th>Criteria</th> <th>Details</th> </tr> </thead> <tbody> <tr> <td>Test temperature</td> <td>20 °C</td> </tr> <tr> <td>Dissolved oxygen</td> <td>At the beginning: oxygen saturated dilution water; at the end: measurement of oxygen (DO ≥ 2 mg/L)</td> </tr> <tr> <td>pH</td> <td>At the beginning: pH 8 of dilution water; after addition of test substance pH not adjusted; at the end: checking whether pH fulfills validity criteria (8.0 ± 0.2)</td> </tr> <tr> <td>Adjustment of pH</td> <td>No.</td> </tr> <tr> <td>Aeration of dilution water</td> <td>Yes. Till saturation.</td> </tr> <tr> <td>Quality/Intensity of irradiation</td> <td>Light intensity: E = 2.5 W/m²</td> </tr> <tr> <td>Photoperiod</td> <td>9 h light/15 h dark</td> </tr> </tbody> </table>	Criteria	Details	Test temperature	20 °C	Dissolved oxygen	At the beginning: oxygen saturated dilution water; at the end: measurement of oxygen (DO ≥ 2 mg/L)	pH	At the beginning: pH 8 of dilution water; after addition of test substance pH not adjusted; at the end: checking whether pH fulfills validity criteria (8.0 ± 0.2)	Adjustment of pH	No.	Aeration of dilution water	Yes. Till saturation.	Quality/Intensity of irradiation	Light intensity: E = 2.5 W/m ²	Photoperiod	9 h light/15 h dark
Criteria	Details																	
Test temperature	20 °C																	
Dissolved oxygen	At the beginning: oxygen saturated dilution water; at the end: measurement of oxygen (DO ≥ 2 mg/L)																	
pH	At the beginning: pH 8 of dilution water; after addition of test substance pH not adjusted; at the end: checking whether pH fulfills validity criteria (8.0 ± 0.2)																	
Adjustment of pH	No.																	
Aeration of dilution water	Yes. Till saturation.																	
Quality/Intensity of irradiation	Light intensity: E = 2.5 W/m ²																	
Photoperiod	9 h light/15 h dark																	
3.4.5	Duration of the test	24 h																
3.4.6	Test parameter	Immobilisation																
3.4.7	Sampling	No information																
3.4.8	Monitoring of TS concentration	No																
3.4.9	Statistics	The EC ₅₀ values were determined graphically based on the experimental results.																
		4 RESULTS																
4.1	Limit Test	No information																
4.1.1	Concentration	-																
4.1.2	Number/ percentage of animals showing adverse effects	-																
4.1.3	Nature of adverse effects	-																
4.2	Results test substance																	
4.2.1	Initial concentrations of test substance	No data																

x

Section A7.4.1.2/02 Acute toxicity to invertebrates**Annex Point IIA7.2***Daphnia magna* STRAUS

4.2.2 Actual concentrations of test substance No analytical monitoring.

4.2.3 Effect data (Immobilisation) EC₅₀, NOEC and EC₁₀₀ values after 24 h

	EC ₅₀	95 % c.l.	EC ₀	EC ₁₀₀
24 h [mg/L]	9714 (n)	-	5102 (n)	>10 000 (n)
48 h [mg/L]				

n-nominal concentrations

4.2.4 Concentration / response curve No data

4.2.5 Other effects No data

4.3 Results of controls No information

4.4 Test with reference substance No information

4.4.1 Concentrations -

4.4.2 Results -

5 APPLICANT'S SUMMARY AND CONCLUSION**5.1 Materials and methods**

The study was conducted according to the draft of the German DIN 38412 part 11: *Daphnia* short-term test (1982). Twenty daphnids ≤ 24 h old were exposed to 2-propanol per concentration step. The test vessels were only loosely covered with filter paper. Based on the short exposure period no significant losses due to volatilisation are assumed. However, analytical monitoring was not performed.

5.2 Results and discussion

Validity criteria	fulfilled	Not fulfilled
Immobilisation of control animals <10%	No data	No data
Control animals not staying at the surface	No data	No data
Concentration of dissolved oxygen in all test vessels >3 mg/l	No data	No data
Concentration of test substance ≥80% of initial concentration during test	No data	No data

The study was conducted according to national standard method. Deviations from this procedure were not reported. Therefore the validity criteria can be considered as fulfilled.

Propan-2-ol was found to be toxic towards *Daphnia magna* in the static test at a concentration of 9714 mg/L nominal (24 h EC₅₀).

No information on dose-response relationship is available.

Although propan-2-ol shows a moderate volatility from aqueous solution (cf. Doc III A3.2.1) the test vessels were only loosely covered with filter paper. But based on the short exposure period (24 h) no significant losses due to volatilisation are assumed. However, analytical monitoring of test substance concentration was not performed.

5.2.1 24 h EC₀ 5102 mg/L (nominal)

Section A7.4.1.2/02 Acute toxicity to invertebrates

Annex Point IIA7.2 *Daphnia magna* STRAUS

5.2.2 24 h EC₅₀ 9714 mg/L (nominal)
5.2.3 24 h EC₁₀₀ >10 000 mg/L (nominal)

5.3 Conclusion

5.3.1 Reliability

5.3.2 Deficiencies

[REDACTED]

[REDACTED]

[REDACTED]

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Use separate "evaluation boxes" to provide transparency as to the comments and views submitted.

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Date 2008/06/30

Materials and Methods

Results and discussion

Conclusion

Reliability

Acceptability

Remarks

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

	COMMENTS FROM ...
Date	<i>Give date of comments submitted</i>
Materials and Methods	<i>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</i>
Results and discussion	<i>Discuss if deviating from view of rapporteur member state</i>
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>
Reliability	<i>Discuss if deviating from view of rapporteur member state</i>
Acceptability	<i>Discuss if deviating from view of rapporteur member state</i>
Remarks	

Section A7.4.1.2/03 **Acute toxicity to invertebrates***Daphnia magna* STRAUS**Annex Point IIA7.2**

		1 REFERENCE
1.1	Reference	<p>Calleja MC, Personne G, Geladi P (1993) The predictive potential of a battery of ecotoxicological tests for human acute toxicity, as evaluated with the first 50 MEIC chemicals. <i>Altern Lab Anim</i> 21, 330-349 (published)</p> <p>Calleja MC, Personne G, Geladi P (1994) Comparative toxicity of the first 50 multicentre evaluation of <i>in vitro</i> cytotoxicity chemicals to aquatic non-vertebrates. <i>Arch Environ Contam Toxicol</i> 26, 69-78 (published)</p>
1.2	Data protection	No
1.2.1	Data owner	-
1.2.2	Criteria for data protection	No data protection claimed
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	Guideline study	Yes. OECD guideline 202 ' <i>Daphnia magna</i> , acute immobilization test and reproduction test' (1984)
2.2	GLP	██████████
2.3	Deviations	No.
		3 MATERIALS AND METHODS
3.1	Test material	Propan-2-ol
3.1.1	Lot/Batch number	-
3.1.2	Specification	Isopropyl alcohol and isopropanol, respectively
3.1.3	Purity	Min. 99.7 %
3.1.4	Composition of Product	Not applicable
3.1.5	Further relevant properties	Due to the volatility of the substance glass vials capped with polyethylene were used in the test. The vials were filled to the brim and covered tightly, thus avoiding any air-space.
3.1.6	Method of analysis	No analytical monitoring.
3.2	Preparation of TS solution for poorly soluble or volatile test substances	Propan-2-ol is indefinitely miscible with water (cf. Doc III A3.5). Based on the measured Henry's Law Constant propan-2-ol is moderately volatile from aqueous solution (cf. Doc III A3.2.1). Therefore, glass vials capped with polyethylene were used in the test. The vials were filled to the brim and covered tightly, thus avoiding any air-space.
3.3	Reference substance	No
3.3.1	Method of analysis for reference substance	-

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Acute toxicity to invertebrates**Section A7.4.1.2/03***Daphnia magna* STRAUS**Annex Point IIA7.2****3.4 Testing procedure**

Dilution water

Criteria	Details
Source	No information
Alkalinity	No information
Hardness	No information
pH	No information
Ca / Mg ratio	No information
Na / K ratio	No information
Oxygen content	No information
Conductance	No information
Holding water different from dilution water	No information

3.4.2 Test organisms

Criteria	Details
Species	<i>Daphnia magna</i>
Strain	No information
Source	No information
Age	No information
Breeding method	No information
Kind of food	No information
Amount of food	No information
Feeding frequency	No information
Pretreatment	No information
Feeding of animals during test	No information

3.4.3 Test system

Criteria	Details
Renewal of test solution	No
Volume of test vessels	No information
Volume/animal	No information
Number of animals/vessel	No information
Number of vessels/ concentration	≥ 3
Test performed in closed vessels due to significant volatility of TS	Yes. Due to the volatility of the substance glass vials capped with polyethylene were used in the test. The vials were filled to the brim and covered tightly, thus avoiding any air-space.

Acute toxicity to invertebrates**Section A7.4.1.2/03***Daphnia magna* STRAUS**Annex Point IIA7.2**

4.2.4	Concentration / response curve	Not available
4.2.5	Other effects	No information
4.3	Results of controls	No information
4.4	Test with reference substance	No information
4.4.1	Concentrations	-
4.4.2	Results	-

5 APPLICANT'S SUMMARY AND CONCLUSION**5.1 Materials and methods**

The study was conducted according to OECD guideline 202 'Daphnia magna, acute immobilization test and reproduction test' (1984). Due to the volatility of the substance glass vials capped with polyethylene were used in the test. The vials were filled to the brim and covered tightly, thus avoiding any air-space. At least three replicates were conducted. Further details are not reported in the publication.

5.2 Results and discussion

Validity criteria	fulfilled	Not fulfilled
Immobilisation of control animals <10%	No data	No data
Control animals not staying at the surface	No data	No data
Concentration of dissolved oxygen in all test vessels >3 mg/l	No data	No data
Concentration of test substance ≥80% of initial concentration during test	No data	No data

Details of the test performance are not reported. Deviations from guideline were not stated. Even though the information is very limited it can be assumed that the validity criteria were fulfilled.

Propan-2-ol was found to be toxic towards *Daphnia magna* in the static test in the range between 9300 to 9550 mg/L (24 h EC₅₀).

No data for the dose-response relationship are available.

Due to the moderate volatility (cf. Doc III A3.2.1) of the substance glass vials capped with polyethylene were used in the test. The vials were filled to the brim and covered tightly, thus avoiding any air-space. Due to this fact significant changes of test substance concentrations during the test duration (24 h) are not to be expected. Analytical monitoring of test substance concentration was not performed. Due to the test design and short exposure time analytical monitoring seems not necessary.

5.2.1	EC ₀	-
5.2.2	24 h EC ₅₀	9307 - 9554 mg/L (24 h immobilisation test)
5.2.3	EC ₁₀₀	-

5.3 Conclusion

5.3.1	Reliability	■
5.3.2	Deficiencies	■

x

x

Section A7.4.1.2/03 **Acute toxicity to invertebrates**
Daphnia magna STRAUS
Annex Point IIA7.2

[REDACTED]

Evaluation by Competent Authorities	
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	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2008/06/30
Materials and Methods	[REDACTED]
Results and discussion	[REDACTED]
Conclusion	[REDACTED]
Reliability	[REDACTED]
Acceptability	[REDACTED]
Remarks	[REDACTED]
	COMMENTS FROM ...
Date	<i>Give date of comments submitted</i>
Materials and Methods	<i>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</i>
Results and discussion	<i>Discuss if deviating from view of rapporteur member state</i>
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>
Reliability	<i>Discuss if deviating from view of rapporteur member state</i>
Acceptability	<i>Discuss if deviating from view of rapporteur member state</i>

Section A7.4.1.2/03 **Acute toxicity to invertebrates**
Daphnia magna STRAUS
Annex Point IIA7.2

Remarks

Section A7.4.1.2/04 Acute toxicity to invertebrates

Annex Point IIA7.2

Crangon crangon

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1 REFERENCE

- 1.1 Reference** Blackman RAA (1974) Toxicity of oil-sinking agents. Mar Pollut Bull 5, 116-118 (published)
- 1.2 Data protection** No
 - 1.2.1 Data owner -
 - 1.2.2 Criteria for data protection No data protection claimed

2 GUIDELINES AND QUALITY ASSURANCE

- 2.1 Guideline study** No. The study was conducted according to the method described by Portmann & Connor (1968) The toxicity of several oil-spill removers to some species of fish and shellfish. Mar Biol 1, 322-329.
- 2.2 GLP** [REDACTED]
- 2.3 Deviations** Yes. In contrast to the toxicity test of Portmann & Connor the test solution was renewed every 6, 12, or 24 h depending on the volatility of the test substance.

3 MATERIALS AND METHODS

- 3.1 Test material** Propan-2-ol
 - 3.1.1 Lot/Batch number -
 - 3.1.2 Specification Isopropyl alcohol
 - 3.1.3 Purity Purity not stated
 - 3.1.4 Composition of Product Not applicable
 - 3.1.5 Further relevant properties
 - 3.1.6 Method of analysis No analytical monitoring
- 3.2 Preparation of TS solution for poorly soluble or volatile test substances** Propan-2-ol is indefinitely miscible with water (cf. Doc III A3.5). Based on the measured Henry's Law Constant propan-2-ol is moderately volatile from aqueous solution (cf. Doc III A3.2.1). Test concentrations were renewed every 6, 12, or 24 h depending on the volatility of the test substance.
- 3.3 Reference substance** No information
 - 3.3.1 Method of analysis for reference substance -

3.4 Testing procedure

Dilution water

Criteria	Details
Source	No information
Alkalinity	No information
Hardness	No information

Section A7.4.1.2/04

Acute toxicity to invertebrates

Annex Point IIA7.2

Crangon crangon

3.4.2 Test organisms

pH	No information
Ca / Mg ratio	No information
Na / K ratio	No information
Oxygen content	No information
Conductance	No information
Holding water different from dilution water	No information
Criteria	Details
Species	<i>Crangon crangon</i>
Strain	No information
Source	No information
Age	No information
Breeding method	No information
Kind of food	No information
Amount of food	No information
Feeding frequency	No information
Pretreatment	No information
Feeding of animals during test	No information

3.4.3 Test system

Criteria	Details
Renewal of test solution	Test concentrations were renewed every 6, 12, or 24 h depending on the volatility of the test substance
Volume of test vessels	No information
Volume/animal	No information
Number of animals/vessel	20
Number of vessels/concentration	No information
Test performed in closed vessels due to significant volatility of TS	No. However, test concentrations were renewed every 6, 12, or 24 h depending on the volatility of the test substance.

3.4.4 Test conditions

Criteria	Details
Test temperature	No information
Dissolved oxygen	No information
pH	No information
Adjustment of pH	No information
Aeration of dilution water	No information

Section A7.4.1.2/04 Acute toxicity to invertebrates**Annex Point IIA7.2***Crangon crangon*

Quality/Intensity of irradiation	No information
Photoperiod	No information

- 3.4.5 Duration of the test 96 h
- 3.4.6 Test parameter mortality
- 3.4.7 Sampling No information
- 3.4.8 Monitoring of TS concentration No
- 3.4.9 Statistics Results were plotted, and 48 h and 96 h values calculated using the method of Litchfield (1949). The ranges given are those from the extreme curves fitted to the upper and lower 95% confidence limits of the plotted values for the estimated time to reach 50% mortality. The curves were fitted by eye.

4 RESULTS**4.1 Limit Test** No information

4.1.1 Concentration -

4.1.2 Number/
percentage of
animals showing
adverse effects -4.1.3 Nature of adverse
effects -**4.2 Results test
substance**4.2.1 Initial
concentrations of
test substance Not stated4.2.2 Actual
concentrations of
test substance No analytical monitoring performed4.2.3 Effect data
(Immobilisation) Data presented below were converted from those given as ppm (v/v) in the report.
48 and 96 h LC₅₀ (including 95 % c.l.)

	LC ₅₀	95 % c.l.	LC ₀	LC ₁₀₀
48 h [mg/L]	1100 (n)	700 - 1530	-	-
96 h [mg/L]	900 (n)	590 - 1300	-	-

n: nominal concentrations

4.2.4 Concentration /
response curve Not available

4.2.5 Other effects No information

4.3 Results of controls Results of the controls were taken into account. No further data provided.

Section A7.4.1.2/04 Acute toxicity to invertebrates

Annex Point IIA7.2

Crangon crangon

4.4 Test with reference substance No information

4.4.1 Concentrations -

4.4.2 Results -

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The study was conducted according to Portmann & Connor (1968) The toxicity of several oil-spill removers to some species of fish and shellfish. Mar Biol 1, 322-329. Solvents used in the toxicity test were renewed every 6, 12, or 24 h depending on the volatility of the test substance. 20 animals per exposure tank were used. No further information provided.

5.2 Results and discussion

Validity criteria	fulfilled	Not fulfilled
Immobilisation of control animals <10%	No data	No data
Control animals not staying at the surface	No data	No data
Concentration of dissolved oxygen in all test vessels >3 mg/l	No data	No data
Concentration of test substance ≥80% of initial concentration during test	No data	No data

The documentation is insufficient and therefore the study is regarded as invalid.

Propan-2-ol was found to be toxic towards *Crangon crangon* in the static test at propan-2-ol concentrations of 1100 (48 h LC₅₀) and 900 mg/L (96 h LC₅₀).

No data for the dose-response relationship are available.

The influence of the moderate volatility (cf. Doc IIIA3.2.1) of the substance is assumed to be negligible due to the semi-static test conditions.

5.2.1 LC₀ -

5.2.2 LC₅₀ 48 h: 1100 mg/L nominal (reported in the report as 1400 ppm v/v)
96 h: 900 mg/L nominal (reported in the report as 1150 ppm v/v)

5.2.3 LC₁₀₀ -

5.3 Conclusion

[Redacted]

5.3.1 Reliability

[Redacted]

x

5.3.2 Deficiencies

[Redacted]

Evaluation by Competent Authorities

Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

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[Redacted]

Section A7.4.1.2/04 Acute toxicity to invertebrates

Annex Point IIA7.2

Crangon crangon

Materials and Methods	[REDACTED]
Results and discussion	[REDACTED]
Conclusion	[REDACTED]
Reliability	[REDACTED]
Acceptability	[REDACTED]
Remarks	[REDACTED]
COMMENTS FROM ...	
Date	<i>Give date of comments submitted</i>
Materials and Methods	<i>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</i>
Results and discussion	<i>Discuss if deviating from view of rapporteur member state</i>
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>
Reliability	<i>Discuss if deviating from view of rapporteur member state</i>
Acceptability	<i>Discuss if deviating from view of rapporteur member state</i>
Remarks	

Section A7.4.1.2 Acute toxicity to invertebrates**Annex Point IIA7.2***Daphnia magna*Official
use only**1 REFERENCE**

- 1.1 Reference** Lilius H, Isomaa B, Holmstrom T (1994) A comparison of 50 reference chemicals to freshly isolated rainbow trout hepatocytes and *Daphnia magna*. *Aquat Toxicol* 30, 47-60 (published)
- Lilius H, Hästabačka T, Isomaa B (1995) A comparison of the toxicity of 30 reference chemicals to *Daphnia magna* and *Daphnia pulex*. *Environ Toxicol Chem* 14, 2085-2088 (published)

- 1.2 Data protection** No
- 1.2.1 Data owner -
- 1.2.2 Criteria for data protection No data protection claimed

2 GUIDELINES AND QUALITY ASSURANCE

- 2.1 Guideline study** Yes. OECD guideline 202 'Daphnia sp., acute immobilisation test and reproduction test' (1993)

2.2 GLP

- 2.3 Deviations** Yes. The toxicity tests were performed in 100-mL polpropylene vessels, with 50 mL liquid in each. Twenty neonates (<24 h) were introduced into vessels containing different concentrations of the test chemical, and the vessels were closed with polyethylene caps.

3 MATERIALS AND METHODS

- 3.1 Test material** Isopropyl alcohol
- 3.1.1 Lot/Batch number -
- 3.1.2 Specification -
- 3.1.3 Purity Reagent grade
- 3.1.4 Composition of Product -
- 3.1.5 Further relevant properties The tests were conducted in test vessels filled to the half and capped with polyethylene to prevent volatilization
- 3.1.6 Method of analysis No analytical monitoring
- 3.2 Preparation of TS solution for poorly soluble or volatile test substances** Propan-2-ol is indefinitely miscible with water (cf. **Doc III A3.5**). Based on the measured Henry's Law Constant propan-2-ol is moderately volatile from aqueous solution (cf. **Doc III A3.2.1**). Test performed in half filled vessels capped with polyethylene to prevent volatilisation.
- 3.3 Reference substance** No information
- 3.3.1 Method of analysis for reference substance No analytical monitoring

3.4 Testing procedure

- 3.4.1 Dilution water

Criteria	Details
----------	---------

Section A7.4.1.2

Acute toxicity to invertebrates

Annex Point IIA7.2

Daphnia magna

3.4.2 Test organisms

Source	SRW according to OECD 1980/1993
Alkalinity	-
Hardness	-
pH	7.6
Ca / Mg ratio	-
Na / K ratio	-
Oxygen content	-
Conductance	-
Holding water different from dilution water	Yes. M4 media according to Elendt and Bias (1990)
Criteria	Details
Species	<i>Daphnia magna</i>
Strain	No information
Source	Department of Biology, University of Turku
Age	<24 h
Breeding method	Breeding in M4 media, 12:12 h light/dark cycle, breeding temperature 20 +/- 2°C
Kind of food	<i>Scenedesmus</i> sp.
Amount of food	-
Feeding frequency	Every second or third day
Pretreatment	No information
Feeding of animals during test	No
Criteria	Details
Renewal of test solution	No
Volume of test vessels	100 mL
Volume/animal	2.5 mL/animal
Number of animals/vessel	20
Number of vessels/concentration	≥3
Test performed in closed vessels due to significant volatility of TS	Yes. Test performed in half filled vessels capped with polyethylene to prevent/minimize volatilisation
Criteria	Details
Test temperature	21 ±1 °C
Dissolved oxygen	No information

3.4.3 Test system

3.4.4 Test conditions

Section A7.4.1.2

Acute toxicity to invertebrates

Annex Point IIA7.2

Daphnia magna

pH	No information
Adjustment of pH	No information
Aeration of dilution water	No
Quality/Intensity of irradiation	No information
Photoperiod	Photoperiod: 12 h dark 12 h light

3.4.5	Duration of the test	24 h	x
3.4.6	Test parameter	Immobility	
3.4.7	Sampling	-	
3.4.8	Monitoring of TS concentration	No	
3.4.9	Statistics	EC ₅₀ values were calculated using regression analysis after linearisation of dose/response curves by logarithmic transformation of the concentration	

4 RESULTS

4.1 Limit Test

No information

4.1.1 Concentration

-

4.1.2 Number/
percentage of
animals showing
adverse effects

-

4.1.3 Nature of adverse
effects

-

**4.2 Results test
substance**

4.2.1 Initial
concentrations of
test substance

No information

4.2.2 Actual
concentrations of
test substance

Concentrations refer to nominal concentration

4.2.3 Effect data
(Immobilisation)

24 h EC₅₀

	EC ₅₀	95 % c.l.	EC ₀	EC ₁₀₀
24 h [mg/L]	6850 (n)	-	-	-
48 h [mg/L]	-	-	-	-

n: nominal

4.2.4 Concentration /
response curve

No information

4.2.5 Other effects

No information

4.3 Results of controls

No information

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 *Daphnia magna*

4.4 Test with reference substance No information

4.4.1 Concentrations -

4.4.2 Results -

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods Yes. OECD guideline 202 '*Daphnia* sp., acute immobilisation test and reproduction test' (1993).

Deviations from guideline: The toxicity tests were performed in 100-mL polpropylene vessels, with 50 mL liquid in each. Twenty neonates (<24 h) were introduced into vessels containing different concentrations of the test chemical, and the vessels were closed with polyethylene cap.

5.2 Results and discussion

Validity criteria	fulfilled	Not fulfilled
Immobilisation of control animals <10%	No data	No data
Control animals not staying at the surface	No data	No data
Concentration of dissolved oxygen in all test vessels >3 mg/l	No data	No data
Concentration of test substance ≥80% of initial concentration during test	No data	No data

The study was conducted according to OECD guideline 202. Deviations from guideline test design were noted. Further deviations were not reported and therefore it can be assumed that the validity criteria were fulfilled.

Propan-2-ol was found to be toxic towards *Daphnia magna* in the static test at a concentration of 6850 mg/L nominal (24 h EC₅₀).

No information is available on the dose-response relationship.

The tests were conducted in test vessels filled to the half and capped with polyethylene to prevent/minimize volatilization.

5.2.1 EC₀ -

5.2.2 24h EC₅₀ 6850 mg/L (nominal)

5.2.3 EC₁₀₀ -

5.3 Conclusion

[Redacted]

5.3.1 Reliability

[Redacted]

5.3.2 Deficiencies

[Redacted]

x

x

Section A7.4.1.2

Acute toxicity to invertebrates

Annex Point IIA7.2

Daphnia magna

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	2008/06/30
Materials and Methods	[REDACTED]
Results and discussion	[REDACTED]
Conclusion	[REDACTED]
Reliability	[REDACTED]
Acceptability	[REDACTED]
Remarks	[REDACTED]
COMMENTS FROM ...	
Date	<i>Give date of comments submitted</i>
Materials and Methods	<i>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</i>
Results and discussion	<i>Discuss if deviating from view of rapporteur member state</i>
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>
Reliability	<i>Discuss if deviating from view of rapporteur member state</i>
Acceptability	<i>Discuss if deviating from view of rapporteur member state</i>
Remarks	

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Section 7.4.1.2/06
Annex Point IIA7.2

Acute toxicity to invertebrates
Daphnia magna STRAUS

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1 REFERENCE

1.1 Reference Hermens J, Canton H, Janssen P, De Jong R (1984) Quantitative structure activity relationship and mixture toxicity studies of chemicals with anaesthetic potency: acute lethal and sublethal toxicity to *Daphnia magna*. *Aquat Toxicol* 5, 143-154 (published)

1.2 Data protection No

1.2.1 Data owner -

1.2.2 Criteria for data protection No data protection claimed

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study No. But the study was conducted according to the Dutch standard method NEN 6501 'Determination of the acute toxicity with *Daphnia magna*' Dutch Standard Organization, Delft (1980).

2.2 GLP [REDACTED]

2.3 Deviations The test duration was 48 h.

3 METHOD

3.1 Test material Propan-2-ol

3.1.1 Lot/Batch number -

3.1.2 Specification Propanol-2

3.1.3 Purity Purity not stated

3.1.4 Composition of Product Not applicable

3.1.5 Further relevant properties -

3.1.6 Method of analysis GC/FID (column: 10% Carbowax 20 M)

3.2 Preparation of TS solution for poorly soluble or volatile test substances Propan-2-ol is indefinitely miscible with water (cf. **Doc III A3.5**). Based on the measured Henry's Law Constant propan-2-ol is moderately volatile from aqueous solution (cf. **Doc III A3.2.1**).

3.3 Reference substance No data

3.3.1 Method of analysis for reference substance -

3.4 Testing procedure

3.4.1 Dilution water

Criteria	Details
Source	Dutch Standard Water according to Canton and Sloof (1982) Toxicity and

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Section 7.4.1.2/06
Annex Point II A7.2

Acute toxicity to invertebrates

Daphnia magna STRAUS

	accumulation studies of Cd (Cd ²⁺) with freshwater organisms of different trophic levels. Ecotoxicol Environ Safe 6, 113-128																						
Salinity	No data																						
Hardness	ca. 1 mmol/L																						
pH	No data																						
Ca / Mg ratio	No data																						
Na / K ratio	No data																						
Oxygen content	No data																						
Conductance	No data																						
TOC	No data																						
Holding water different from dilution water	No data																						
3.4.2 Test organisms	<table border="1"> <thead> <tr> <th>Criteria</th> <th>Details</th> </tr> </thead> <tbody> <tr> <td>Species</td> <td><i>Daphnia magna</i></td> </tr> <tr> <td>Strain / Clone</td> <td>Not stated</td> </tr> <tr> <td>Source</td> <td>No data</td> </tr> <tr> <td>Age</td> <td>< 2 d</td> </tr> <tr> <td>Breeding method</td> <td>No data</td> </tr> <tr> <td>Kind of food</td> <td>none</td> </tr> <tr> <td>Amount of food</td> <td>No data</td> </tr> <tr> <td>Feeding frequency</td> <td>No data</td> </tr> <tr> <td>Pretreatment</td> <td>No data</td> </tr> <tr> <td>Feeding of animals during test</td> <td>No data</td> </tr> </tbody> </table>	Criteria	Details	Species	<i>Daphnia magna</i>	Strain / Clone	Not stated	Source	No data	Age	< 2 d	Breeding method	No data	Kind of food	none	Amount of food	No data	Feeding frequency	No data	Pretreatment	No data	Feeding of animals during test	No data
Criteria	Details																						
Species	<i>Daphnia magna</i>																						
Strain / Clone	Not stated																						
Source	No data																						
Age	< 2 d																						
Breeding method	No data																						
Kind of food	none																						
Amount of food	No data																						
Feeding frequency	No data																						
Pretreatment	No data																						
Feeding of animals during test	No data																						
3.4.3 Handling of offspring	Not reported																						
3.4.4 Test system	<table border="1"> <thead> <tr> <th>Criteria</th> <th>Details</th> </tr> </thead> <tbody> <tr> <td>Test type</td> <td>Static</td> </tr> <tr> <td>Renewal of test solution</td> <td>no</td> </tr> <tr> <td>Volume of test vessels</td> <td>1 L</td> </tr> <tr> <td>Volume/animal</td> <td>40 mL/animal</td> </tr> <tr> <td>Number of animals/vessel</td> <td>25</td> </tr> <tr> <td>Number of vessels/ concentration</td> <td>2</td> </tr> </tbody> </table>	Criteria	Details	Test type	Static	Renewal of test solution	no	Volume of test vessels	1 L	Volume/animal	40 mL/animal	Number of animals/vessel	25	Number of vessels/ concentration	2								
Criteria	Details																						
Test type	Static																						
Renewal of test solution	no																						
Volume of test vessels	1 L																						
Volume/animal	40 mL/animal																						
Number of animals/vessel	25																						
Number of vessels/ concentration	2																						

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Section 7.4.1.2/06
Annex Point IIA7.2

Acute toxicity to invertebrates

Daphnia magna STRAUS

3.4.5	Test conditions	Test performed in closed vessels due to significant volatility of TS	No data
		Criteria	Details
		Test temperature	22 ± 1 °C
		Dissolved oxygen	No data
		pH	No data
		Adjustment of pH	No data
		Aeration of dilution water	No data
		Quality/Intensity of irradiation	No data
		Photoperiod	No data

- 3.4.6 Duration of the test 48 h
- 3.4.7 Test parameter Immobility
- 3.4.8 Examination / Sampling Not explicitly mentioned. Test procedure is described in the NEN report 6501.
- 3.4.9 Monitoring of TS concentration Yes. Effect values are based on nominal quantities.
- 3.4.10 Statistics Estimation of IC50 values by logit transformation according to Brown (1978)

4 RESULTS

- 4.1 Range finding test** No data
- 4.1.1 Concentrations -
- 4.1.2 Number/percentage of animals showing adverse effects -
- 4.1.3 Nature of adverse effects -
- 4.2 Results test substance**
- 4.2.1 Initial concentrations of test substance No data
- 4.2.2 Actual concentrations of test substance Actual concentrations were determined during the test by gaschromatographic analysis (no further information)
- 4.2.3 Effect data 48 d IC₅₀ = 2285 mg/L
No further information

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Section 7.4.1.2/06
Annex Point II A7.2

Acute toxicity to invertebrates
Daphnia magna STRAUS

4.2.4 Concentration / response curve Not available

4.2.5 Other effects No data

4.3 Results of controls No data

4.4 Test with reference substance No data

4.4.1 Concentrations -

4.4.2 Results -

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods The study was conducted according to the Dutch standard method NEN 6501 (1980).

The test was performed in 1 L flasks and 25 daphnids (tests conducted in duplicate: 50 daphnids per concentration). Actual concentration of the test substance was measured analytically. No further information is given.

5.2 Results and discussion In the acute study with *Daphnia magna* a 48 h IC₅₀ = 2285 mg/L (4.58 µmol/L) was determined in respect to immobilisation. No information is provided about the mortality of parent animals at test termination.

The study was conducted according to a national standard method.

No information on dose-response relationship is given.

Based on the experimentally determined Henry's Law constant (cf. **Doc III A3.2.1**) a moderate volatilisation from aqueous solution is to be expected.

5.2.1 NOEC -

5.2.2 LOEC -

5.2.3 EC₅₀ (EC_x) 48 h IC₅₀ = 2285 mg/L

5.3 Conclusion

[REDACTED]

5.3.1 Reliability

[REDACTED]

5.3.2 Deficiencies

[REDACTED]

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Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	2008/12/05
Materials and Methods	[REDACTED]
Results and discussion	[REDACTED]
Conclusion	[REDACTED]
Reliability	[REDACTED]
Acceptability	[REDACTED]
Remarks	[REDACTED]
COMMENTS FROM ... (specify)	
Date	<i>Give date of comments submitted</i>
Materials and Methods	<i>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</i>
Results and discussion	<i>Discuss if deviating from view of rapporteur member state</i>
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>
Reliability	<i>Discuss if deviating from view of rapporteur member state</i>
Acceptability	<i>Discuss if deviating from view of rapporteur member state</i>
Remarks	

Section A7.4.1.3/01 **Growth inhibition test on algae**
 Annex Point IIA7.3 *Selenastrum capricornutum*

substance

3.4 Testing procedure

3.4.1 Culture medium Culture medium indicated in the OECD guideline

3.4.2 Test organisms	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]

3.4.3 Test system	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]

3.4.4 Test conditions	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]

3.4.5 Duration of the test [REDACTED]

3.4.6 Test parameter [REDACTED]

Section A7.4.1.3/01 Growth inhibition test on algae

Annex Point IIA7.3

Selenastrum capricornutum

- 3.4.7 Sampling [Redacted]
- 3.4.8 Monitoring of TS concentration [Redacted]
- 3.4.9 Statistics [Redacted]

4 RESULTS

- 4.1 Limit Test** Not performed
- 4.1.1 Concentration Not applicable
- 4.1.2 Number/percentage of animals showing adverse effects Not applicable

4.2 Results test substance

- 4.2.1 Initial concentrations of test substance [Redacted] X
- 4.2.2 Actual concentrations of test substance [Redacted]
- 4.2.3 Growth curves **Growth curve of *Selenastrum capricornutum*** X



- 4.2.4 Concentration / response curve [Redacted]
- 4.2.5 Cell concentration data **Inhibition percentage after 72 hours:**
[Redacted] [Redacted]

Section A7.4.1.3/01

Growth inhibition test on algae

Annex Point IIA7.3

Selenastrum capricornutum

[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]

4.2.6 Effect data
(cell multiplication
inhibition)

[REDACTED]

4.2.7 Other observed
effects

[REDACTED]

4.3 Results of controls cf. 4.2.3

4.4 Test with
reference
substance Potassium dichromate

4.4.1 Concentrations No data

4.4.2 Results 48 h E₀C₅₀ = 0.369 mg/L

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and
methods

[REDACTED]

5.2 Results and
discussion

[REDACTED]

x

[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]

5.2.1 NOE_rC

[REDACTED]

5.2.2 E_rC₅₀

[REDACTED]

Section A7.4.1.3/01 Growth inhibition test on algae

Annex Point IIA7.3

Selenastrum capricornutum

5.3 Conclusion

5.3.1 Reliability

5.3.2 Deficiencies

[REDACTED]

[REDACTED]

[REDACTED]



Evaluation by Competent Authorities

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EVALUATION BY RAPPOREUR MEMBER STATE	
Date	2014/02/13
Materials and Methods	[REDACTED]
Results and discussion	[REDACTED]
Conclusion	[REDACTED]
Reliability	[REDACTED]
Acceptability	[REDACTED]
Remarks	[REDACTED]

COMMENTS FROM ...	
Date	<i>Give date of comments submitted</i>
Materials and Methods	<i>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</i>
Results and discussion	<i>Discuss if deviating from view of rapporteur member state</i>
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>
Reliability	<i>Discuss if deviating from view of rapporteur member state</i>
Acceptability	<i>Discuss if deviating from view of rapporteur member state</i>
Remarks	

Section A7.4.1.3/02 Growth inhibition test on algae**Annex Point IIA7.3**

		1 REFERENCE
1.1	Reference	Bringmann G, Kuehn R (1977) Grenzwerte der Schadwirkung wassergefährdender Stoffe gegen Bakterien (<i>Pseudomonas putida</i>) und Grünalgen (<i>Scenedesmus quadricauda</i>) im Zellvermehrungshemmtest. Z Wasser Abwasser-Forsch 10, 87-98 (published) Bringmann G, Kuehn R (1978) Grenzwerte der Schadwirkung wassergefährdender Stoffe gegen Blaualgen (<i>Microcystis aeruginosa</i>) und Grünalgen (<i>Scenedesmus quadricauda</i>) im Zellvermehrungshemmtest. Vom Wasser 50, 45-60 (published) Bringmann G, Kuehn R (1980) Comparison of the toxicity thresholds of water pollutants to bacteria, algae, and protozoa in the cell multiplication inhibition test. Water Res 14, 231-241 (published)
1.2	Data protection	No
1.2.1	Data owner	-
1.2.2	Criteria for data protection	No data protection claimed
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	Guideline study	No. Not available at the time the study was conducted. But the test procedure used in this study was comparable to a national standard method.
2.2	GLP	
2.3	Deviations	-
		3 MATERIALS AND METHODS
3.1	Test material	Propan-2-ol
3.1.1	Lot/Batch number	-
3.1.2	Specification	Isopropanol and 2-propanol
3.1.3	Purity	No data
3.1.4	Composition of Product	Not applicable
3.1.5	Further relevant properties	-
3.1.6	Method of analysis	No analytical monitoring
3.2	Preparation of TS solution for poorly soluble or volatile test substances	Propan-2-ol is indefinitely miscible with water (cf. Doc III A3.5). Based on the measured Henry's Law Constant propan-2-ol is moderately volatile from aqueous solution (cf. Doc III A3.2.1). Therefore, the test was conducted in culture tubes stoppered with metal caps
3.3	Reference substance	No information
3.3.1	Method of analysis for reference substance	-

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Section A7.4.1.3/02 Growth inhibition test on algae**Annex Point IIA7.3****3.4 Testing procedure**

3.4.1 Culture medium

sodium nitrate	496 mg
dipotassium hydrogen phosphate, anhydrous	39 mg
magnesium sulphate	75 mg
calcium chloride	36 mg
sodium metasilicate	40 mg
sodium carbonate, anhydrous	58 mg
citric acid	3 mg
iron citrate	3 mg
disodium salt of EDTA	10 mg

The aforementioned nutrients were dissolved in double-distilled water. 10 mL of trace elements solution was added. Double-distilled water was used to complete the solution to 1 L. The pH was adjusted to pH 7.0 using Na₂CO₃ solution.

3.4.2 Test organisms

Criteria	Details
Species	Algae
Strain	<i>Scenedesmus quadricauda</i>
Source	Own culture
Laboratory culture	Yes
Method of cultivation	Stock cultures stored in 20 mL nutrient solution in Erlenmeyer flasks stoppered with metal caps, on a white surface protected against daylight and exposed to constant lightning by luminescent warm white tubes at 60 cm distance from each other, at 27 °C and a relative humidity of 50%; fresh stock cultures were prepared continuously at 10 days' intervals; the algae were separated from the culture solution by membrane filtration
Pretreatment	No information
Initial cell concentration	No information on initial cell concentration, but the concentration was adjusted based on the extinction value (turbidity measurement)

3.4.3 Test system

Criteria	Details
Volume of culture flasks	10 mL
Culturing apparatus	Kapsenberg tube
Light quality	Constant lightning by two luminescent warm white tubes at 60 cm distance

Section A7.4.1.3/02 Growth inhibition test on algae**Annex Point IIA7.3**

		from each other												
	Procedure for suspending algae	Shaking once a day												
	Number of vessels/ concentration	3 tubes												
	Test performed in closed vessels due to significant volatility of TS	Yes. The test was conducted in culture tubes stoppered with metal caps												
3.4.4	Test conditions	<table border="1"> <thead> <tr> <th>Criteria</th> <th>Details</th> </tr> </thead> <tbody> <tr> <td>Test temperature</td> <td>27 °C</td> </tr> <tr> <td>pH</td> <td>No information</td> </tr> <tr> <td>Aeration of dilution water</td> <td>No information</td> </tr> <tr> <td>Light intensity</td> <td>No information</td> </tr> <tr> <td>Photoperiod</td> <td>Continuous lightning</td> </tr> </tbody> </table>	Criteria	Details	Test temperature	27 °C	pH	No information	Aeration of dilution water	No information	Light intensity	No information	Photoperiod	Continuous lightning
Criteria	Details													
Test temperature	27 °C													
pH	No information													
Aeration of dilution water	No information													
Light intensity	No information													
Photoperiod	Continuous lightning													
3.4.5	Duration of the test	7 or 8 days (according to the literature the EC ₃ was determined after 7 or 8 days of exposure; the information is different in the above cited literatures)												
3.4.6	Test parameter	Cell multiplication inhibition (biomass)												
3.4.7	Sampling	After termination of the test												
3.4.8	Monitoring of TS concentration	No analytical monitoring												
3.4.9	Statistics	EC ₃ described as Toxicity Threshold (= TT) determined graphically based on experimental results.												

4 RESULTS

4.1	Limit Test	No information
4.1.1	Concentration	-
4.1.2	Number/ percentage of animals showing adverse effects	-
4.2	Results test substance	
4.2.1	Initial concentrations of test substance	No information
4.2.2	Actual concentrations of test substance	No analytical monitoring
4.2.3	Growth curves	No information
4.2.4	Concentration / response curve	Not available

Section A7.4.1.3/02 Growth inhibition test on algae

Annex Point IIA7.3

- 4.2.5 Cell concentration data Not reported in the publication.
- 4.2.6 Effect data (cell multiplication inhibition) 7 d/8 d E_bC₃ = 1800 mg/L (nominal)
- 4.2.7 Other observed effects No information
- 4.3 Results of controls** No information
- 4.4 Test with reference substance** No information
- 4.4.1 Concentrations -
- 4.4.2 Results -

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods The study was conducted according to Bringmann & Kuehn (1977, 1978, and 1980); the procedure is comparable to a national standard method. In the cell multiplication inhibition test the 7 d/8 d EC₃ described as Toxicity Threshold (=TT) was determined in Kapsenberg culture tubes via measurement of turbidity. No information is given whether the cultures were in the exponential growth phase during the test period. The test was conducted in Kapsenberg tubes stoppered with metal caps. Analytical monitoring was not performed.

5.2 Results and discussion

	fulfilled	Not fulfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	No data	No data
Concentration of test substance ≥80% of initial concentration during test	No data	No data

In a cell multiplication inhibition test according to Bringmann & Kuehn a 7 d/8 d E_bC₃ = 1800 mg/L (nominal) was determined. In general the study is very well documented. However, it is not stated whether the cultures were in the exponential growth phase during the test period.

2-Propanol shows a very low toxicity towards *Scenedesmus quadricauda* in the cell multiplication inhibition test (7d/8d E_bC₃= 1800 mg/L).

No information on dose-response relationship is available.

The influence of the moderate volatility (cf. **Doc IIIA3**) of the substance is assumed to be negligible due to the fact that the test tubes were stoppered with metal caps.

- 5.2.1 NOE_bC 7d/8d E_bC₃: 1800 mg/L (nominal)
- 5.2.2 E_{t50} -
- 5.2.3 E_bC₅₀

5.3 Conclusion

[Redacted]

- 5.3.1 Reliability

█

x