This document has been prepared by the competent authority and does not necessarily represent the participant`s opinion.

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	*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 A7.4.1.3/01 Growth inhibition test on algae

Annex Point IIA7.3 *Selenastrum capricornutum*

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted](1998) Final Report, Growth Inhibition Test Using Selenastrum capricornutum to 2-Propanol. [redacted] [redacted] 2007 Chemical Risk Information Platform (CHRIP) Total Search System for Chemical Substances: 2-Propanol; [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**	Yes. [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]
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
3.3.1	Method of analysis for reference	No data

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]
3.4.3	Test system	[redacted]
3.4.4	Test conditions	[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*** [redacted]	x
4.2.4	Concentration / response curve	[redacted]	
4.2.5	Cell concentration data	**Inhibition percentage after 72 hours**: [redacted]	x

Section A7.4.1.3/01 Growth inhibition test on algae

Annex Point IIA7.3 *Selenastrum capricornutum*

[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 E_bC_{50} = 0.369 mg/L

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	[redacted]
5.2	**Results and discussion**	[redacted] x
5.2.1	NOE_rC	[redacted]
5.2.2	E_rC_{50}	[redacted]

Section A7.4.1.3/01 Growth inhibition test on algae

Annex Point IIA7.3 *Selenastrum capricornutum*

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	2014/02/13
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 A7.4.1.3/02 Growth inhibition test on algae

Annex Point IIA7.3

Official use only

		1 REFERENCE
1.1	**Reference**	Bringmann G, Kuehn R (1977) Grenzwerte der Schadwirkung wassergefährdender Stoffe gegen Bakterien (*Pseudomonas putida*) und Grünalgen (*Scenedesmus quadricauda*) im Zellvermehrungshemmtest. Z Wasser Abwasser-Forsch 10, 87-98 (published) Bringmann G, Kuehn R (1978) Grenzwerte der Schadwirkung wassergefährdender Stoffe gegen Blaualgen (*Microcystis aeruginosa*) und Grünalgen (*Scenedesmus quadricauda*) 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**	[redacted]
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	-

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-destilled 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 Na_2CO_3 solution.

3.4.2 Test organisms

Criteria	**Details**
Species	Algae
Strain	*Scenedesmus quadricauda*
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	**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	7or 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_bC_3 = 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 EC_3 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_bC_3 = 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 *Scendesmus quadricauda* in the cell multiplication inhibition test (7d/8d E_bC_3= 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_bC_3: 1800 mg/L (nominal)
5.2.2	E_{r50}	-
5.2.3	E_bC_{50}	
5.3	**Conclusion**	[redacted]
5.3.1	Reliability	[redacted] x

Section A7.4.1.3/02 Growth inhibition test on algae

Annex Point IIA7.3

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	2008/07/04
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*

Section A7.4.1.3/02 Growth inhibition test on algae

Annex Point IIA7.3

Remarks

Section A7.4.1.3/03 Growth inhibition test on algae

Annex Point IIA7.3

Official use only

1 REFERENCE

1.1	**Reference**	Bringmann G, Kuehn R (1978) Grenzwerte der Schadwirkung wassergefährdender Stoffe gegen Blaualgen (*Microcystis aeruginosa*) und Grünalgen (*Scenedesmus quadricauda*) im Zellvermehrungshemmtest. Vom Wasser 50, 45-60 (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**	[redacted]
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, respectively
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**	2-Propanol is indefinitely miscible with water (**cf. Doc III A3.5**). Based on the measured Henry's Law Constant 2-propanol is moderately volatile from aqueous solution (**cf. Doc IIIA3.2.1**). 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	-
3.4	**Testing procedure**	
3.4.1	Culture medium	

sodium nitrate	496 mg
dipotassium hydrogen phosphate, anhydrous	39 mg
magnesium sulphate	75 mg

Section A7.4.1.3/03 Growth inhibition test on algae

Annex Point IIA7.3

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-destilled 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 Na_2CO_3 solution.

3.4.2 Test organisms

Criteria	**Details**
Species	*Microcystis aeruginosa* = Blue-green algae (bacteria)
Strain	No data
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 from each other
Procedure for suspending algae	Shaking once a day
Number of vessels/ concentration	3 tubes

Section A7.4.1.3/03 Growth inhibition test on algae

Annex Point IIA7.3

		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	**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	8 days (according to the literature the EC_3 described as Toxicity Threshold (=TT) was determined after 8 days of exposure)	x
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 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	
4.2.5	Cell concentration data	Not reported in the publication.	
4.2.6	Effect data (cell multiplication inhibition)	8 d E_bC_3 = 1000 mg/L (nominal)	
4.2.7	Other observed effects	No information	

Section A7.4.1.3/03 Growth inhibition test on algae

Annex Point IIA7.3

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 & Kühn (1978). In the cell multiplication inhibition test the 8 d EC_3 desscribed as Toxicity Threshold (= TT) for *Microcystis aeruginosa* 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

Propan-2-ol shows a very low toxicity towards *Microcystis aeruginosa* in the cell multiplication inhibition test (8d E_bC_3= 1000 mg/L).

	fulfilled	**Not fullfilled**
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**

For *Microcystis aeruginosa* an 8 d E_bC_3 = 1000 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. Based on discussions of results obtained with this test-system within the OECD HPV Chemicals Programme, the results for *Microcystis aeruginosa* are accepeted as valid, because blue-green algae generally grow slower compared to green algae.

No information on dose-response relationship is available.

The influence of the moderate volatility (cf. Doc IIIA) 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	E_bC_3: 1000 mg/L (nominal)
5.2.2	E_{r50}	-
5.2.3	E_bC_{50}	
5.3	**Conclusion**	[redacted]
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	[redacted]

Section A7.4.1.3/03 Growth inhibition test on algae

Annex Point IIA7.3

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2008/07/04
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 A7.4.1.3/04 Growth inhibition test on algae

Annex Point IIA7.3

Official use only

		1 REFERENCE
1.1	**Reference**	Hsieh SH, Tsai KP, Chen CY (2006) The combined toxic effects of nonpolar narcotic chemicals to *Pseudokirchneriella subcapitata*. Water Research 40, 1957-1964 (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, US EPA (1996) OPPTS 850.5400 Algal toxicity (For more detailed information on the test system it is referred to Lin et al. (2005) A novel algal toxicity testing technique for assessing the toxicity of both metallic and organic toxicants. Water research 39, 1869-1877)
2.2	**GLP**	[redacted]
2.3	**Deviations**	Yes, test was run for 48 h instead of 96 h.
		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	99% (Reagen grade)
3.1.4	Composition of Product	Not applicable
3.1.5	Further relevant properties	-
3.1.6	Method of analysis	HPLC analysis of stock solution
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	2-Propanol is indefinitely miscible with water (**cf. Doc III A3.5**). Based on the measured Henry's Law Constant 2-propanol is moderately volatile from aqueous solution (**cf. Doc IIIA3.2.1**). Test was performed in 300 mL BOD bottles, completety filled with no head space left.
3.3	**Reference substance**	No information
3.3.1	Method of analysis for reference substance	-
3.4	**Testing procedure**	
3.4.1	Culture medium	Growth medium as decribed by US EPA 1996 NaNO3 : 12.75 mg/L, K2HPO4: 0.52 mg/L, EDTA: 30 µg/L
3.4.2	Test organisms	

Criteria	Details
Species	*Pseudokirchneriella subcapitata* (former scientific name: *Selenastrum*

Section A7.4.1.3/04 Growth inhibition test on algae

Annex Point IIA7.3

			capricornutum)
		Strain	Not specified
		Source	Not specified
		Laboratory culture	Yes
		Method of cultivation	Cultured in 4-L transparent chemostat incubator. Growth medium was supplied continuously. Temperature: 24+-1°C, light intensity: 65 µE m^{-2} s^{-1} (+-10%) Dilution rate 0.25/d
		Pretreatment	No information
		Initial cell concentration	15000 cells/mL
3.4.3	Test system	**Criteria**	**Details**
		Volume of culture flasks	300 mL
		Culturing apparatus	BOD bottles placed on orbital shaker at 100 rpm
		Light quality	Light intensity: 65 µE m^{-2} s^{-1} (+-10%)
		Procedure for suspending algae	orbital shaker at 100 rpm
		Number of vessels/ concentration	3 replicates
		Test performed in closed vessels due to significant volatility of TS	Yes. BOD bottels
3.4.4	Test conditions	**Criteria**	**Details**
		Test temperature	24 +- 1 °C
		pH	No information
		Aeration of dilution water	No, dilution water was stripped by nitrogen gas to reduce dissolved oxygen level.
		Light intensity	65 µE m^{-2} s^{-1} (+-10%)
		Photoperiod	No information (14 h light/10 h dark, according to guideline)
3.4.5	Duration of the test	48 h	
3.4.6	Test parameter	Algal growth rate based on cell density measured by electronic particle counter (growth rate), dissolved oxygen production	
3.4.7	Sampling	After termination of the test	
3.4.8	Monitoring of TS concentration	Yes, stock solution was analysed by HPLC	
3.4.9	Statistics	EC_{50} determined by probit analysis	

Section A7.4.1.3/04 Growth inhibition test on algae

Annex Point IIA7.3

		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	3500-14000 mg/L	
4.2.2	Actual concentrations of test substance	Stock solution was analysed by HPLC	
4.2.3	Growth curves	Not given in publication	
4.2.4	Concentration / response curve	Not given in publication	
4.2.5	Cell concentration data	Not given in publication	
4.2.6	Effect data (cell multiplication inhibition)	48 h $E_{\mu}C_{50}$ = 10500 (95% CI: 9780-11300) mg/L (nominal) 48 h $E_{DO}C_{50}$ = 8040 (95% CI: 6530-10350) mg/L (nominal) DO: dissolved oxygen	
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 US EPA OPPTS 850.5400 Guideline. The test was performed in 300 mL BOD Bottles Analytical monitoring was performed in stock solution. EC50 for both dissolved oxygen and growth rate was determined by probit analysis.	X
5.2	**Results and discussion**	Propan-2-ol shows a very low toxicity towards *Pseudokirchneriella subcapitata* in the cell multiplication inhibition test (48 h $E_{\mu}C_{50}$= 10500 mg/L).	X

	fulfilled	**Not fullfilled**
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**

Section A7.4.1.3/04 Growth inhibition test on algae

Annex Point IIA7.3

		In general the study is well documented. No information on dose-response relationship is available. The influence of the moderate volatility (cf. Doc IIIA) of the substance is assumed to be negligible due to the fact that the BOD bottles were closed and filled completey.	
5.2.1	NOEC	Not reported	
5.2.2	E_rC_{50}	10500 mg/L (48 h)	
5.2.3	E_bC_{50}		
5.3	**Conclusion**	[redacted]	x
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	2008/09/22 / revised version: 2014/01/15
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 A7.4.1.3/05 Annex Point IIA7.3	Growth inhibition test on algae	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	Technically not feasible [ ] Scientifically unjustified [X]	
Limited exposure [ ]	Other justification [ ]	
Detailed justification:	[redacted]	
References:	[redacted]	

Section A7.4.1.3/05 Annex Point IIA7.3	Growth inhibition test on algae
	[redacted]
Undertaking of intended data submission [ ]	Not applicable, no study is planned.
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2008/09/24

Section A7.4.1.3/05 Annex Point IIA7.3	Growth inhibition test on algae
Evaluation of applicant's justification	[redacted]
Conclusion	[redacted]
Remarks	[redacted]

	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.4.1.3/06 Growth inhibition test on algae

Annex Point IIA7.3

Official use only

1 REFERENCE

1.1	**Reference**	Cho C-W, Jeon Y-C, Pham TPT, Vijayaraghava K, Yun Y-S (2008) The ecotoxicity of ionic liquids and traditional organic solvents on microalga *Selenastrum capricornutum*. Ecotoxicol. Environ. Safety 71, 166-171 (published) [redacted] (2013) Estimation of the EC10 value from the algal test published by Cho et al. 2008 [redacted]
1.2	**Data protection**	No
1.2.1	Data owner	Data published
1.2.2	Criteria for data protection	No data protection claimed

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes OECD guideline 201 ("freshwater alga and cyanobacteria, Growth Inhibition" (2002) OPPTS 850.5400 "Algal Toxicity, Tiers I and II"
2.2	**GLP**	[redacted]
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	Propan-2-ol
3.1.1	Lot/Batch number	No data
3.1.2	Specification	2-Propanol, purchased from Sigma-Aldrich
3.1.3	Purity	> 99.5 %
3.1.4	Composition of Product	Not applicable
3.1.5	Further relevant properties	No data
3.1.6	Method of analysis	Not performed
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	Not applicable
3.4	**Testing procedure**	
3.4.1	Culture medium	No data
3.4.2	Test organisms	

Criteria	Details

Section A7.4.1.3/06 Growth inhibition test on algae

Annex Point IIA7.3

		Species	*Selenastrum capricornutum*
		Strain	ATCC-22662
		Source	National Institute Environmental Research, Korea
		Laboratory culture	Yes
		Method of cultivation	Cultivated in 250 ml Erlenmeyer flasks, containing 200 ml sterilized nitrate-enriched BBM medium prepared in triple distilled water, to avoid nitrogen limitation during the high-density culture. The culture flask was agitated on a shaker at 170 rpm, and bubbled with air (1 vvm), without sparger. Light was continuously supplied, with an average of 30 ± 5 µE m^{-2} s^{-1}, using warm-white fluorescent located on top of the shaker. All the flasks were maintained in the shaker incubator at 25 ± 5 °C for 7 days.
		Pretreatment	None
		Initial cell concentration	No data
3.4.3	Test system	**Criteria**	**Details**
		Volume of culture flasks	250 ml Erlenmeyer flasks, fill volume 60 mL
		Culturing apparatus	Shaker incubator at 170 rpm
		Light quality	Warm-white fluorescent tubes
		Procedure for suspending algae	Shaking
		Number of vessels/ concentration	2 (test substance) 3 (control)
		Test performed in closed vessels due to significant volatility of TS	No data
3.4.4	Test conditions	**Criteria**	**Details**
		Test temperature	25 °C
		pH	No data
		Aeration of dilution water	No
		Light intensity	30 ± 5 µE m^{-2} s^{-1}
		Photoperiod	Continuous illumination
3.4.5	Duration of the test	96 hours	
3.4.6	Test parameter	Dry cell weight. Optical density of the algal biomass was estimated at 438 nm using a spectrophotometer. Dry cell weight (g/L) = 0.1329 x	

Section A7.4.1.3/06 Growth inhibition test on algae

Annex Point IIA7.3

		optical density
3.4.7	Sampling	No data
3.4.8	Monitoring of TS concentration	No
3.4.9	Statistics	Average values from duplicate determinations
		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	Range 1.26 mM – 0.1 M 75 - 6000 mg/L (calculated by the applicants)
4.2.2	Actual concentrations of test substance	Not applicable, as no analysis was performed
4.2.3	Growth curves	[redacted]
4.2.4	Concentration / response curve	[redacted]

Section A7.4.1.3/06 Growth inhibition test on algae

Annex Point IIA7.3

[redacted]

4.2.5	Cell concentration data	No data	
4.2.6	Effect data (cell multiplication inhibition)	Data published: [redacted]	x
4.2.7	Other observed effects	No data	
4.3	**Results of controls**	Not applicable, as no reference substance was tested	
4.4	**Test with reference substance**	Not performed	
4.4.1	Concentrations	Not applicable	
4.4.2	Results	Not applicable	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	[redacted]
5.2	**Results and discussion**	[redacted]
		[redacted]

Section A7.4.1.3/06 Growth inhibition test on algae

Annex Point IIA7.3

[redacted]

5.2.1	NOE_rC	No data, [redacted]	
5.2.2	E_{r50}	[redacted]	
5.2.3	E_bC_{50}	No data	
5.3	**Conclusion**	[redacted]	
5.3.1	Reliability	[redacted]	
5.3.2	Deficiencies	[redacted]	x

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 ...

Section A7.4.1.3/06 Growth inhibition test on algae

Annex Point IIA7.3

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 A7.4.1.4/01 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	Bringmann G, Kuehn R (1977) Grenzwerte der Schadwirkung wassergefährdender Bakterien (*Pseudomonas putida*) und Grünalgen (*Scenedesmus quadricauda*) im Zellvermehrungshemmtest. Z Wasser Abwasser-Forschung 10, 87-98 (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. No guidelines available at the time the study was conducted.	
2.2	**GLP**	[redacted]	
2.3	**Deviations**	Not applicable.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Propan-2-ol	x
3.1.1	Lot/Batch number	-	
3.1.2	Specification	Isopropanol and 2-propanol, respectively	
3.1.3	Purity	Not stated	
3.1.4	Composition of Product	Not applicable	
3.1.5	Further relevant properties	The tests were conducted in Erlenmeyer flasks stoppered with cotton-lined plastic caps.	
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**). Aqueous solutions of known test substance concentrations were prepared in Erlenmeyer flasks. The flasks were stoppered with cotton-lined plastic caps.	
3.3	**Reference substance**	No data.	x
3.3.1	Method of analysis for reference substance	-	
3.4	**Testing procedure**		
3.4.1	Culture medium	Nutrient medium for stock and preliminary cultures (dissolved in 1 L double-distilled water)	

Section A7.4.1.4/01 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

$NaNO_3$	1.06 g
K_2HPO_4, anhydrous	0.6 g
KH_2PO_4	0.3 g
$MgSO_4$ x 7 H_2O	0.2 g
Glucose	10 g
Difco Bacto agar	18 g
$FeSO_4$ x 7 H_2O	0.01 g
Trace elements solution	1.5 mL

Trace elements (in g per liter)

$Al_2(SO_4)$ x 18 H_2O	0.055
KJ	0.028
KBr	0.028
TiO_2	0.055
$SnCl_2$ x 2 H_2O	0.028
LiCl	0.028
$MnCl_2$ x 4 H_2O	0.389
H_3BO_3	0.614
$ZnSO_4$ x 7 H_2O	0.055
$CuSO_4$ x 5 H_2O	0.055
$NiSO_4$ x 6 H_2O	0.059
$Co(NO3)_2$ x 6 H_2O	0.055

Vitamine

D-Biotin	0.2 mg
Nicotinic acid	2 mg
Thiamine HCl	1 mg
p-aminobenzoic acid	1 mg
D-Panthotenic acid Na salt	0.5 mg
Pyridoxamine dihydrochloride	5 mg
Vitamin B_{12}	2 mg
Double-distilled water	100 mL

Stock solution I (dissolved in 1 l double-distilled water)

Glucose	20 g
NaNO3	4.24 g

Section A7.4.1.4/01 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

K_2HPO_4, anhydrous	2.4 g
KH_2PO_4	1.2 g
Trace elements solution	30 mL

Stock solution II (dissolved in 1 L double-distilled water)

$FeSO_4$ x 7 H_2O	0.2 g
$MgSO_4$ x 7 H_2O	4 g

3.4.2 Inoculum / test organism

Criteria	**Details**
Nature	Bacteria
Species	*Pseudomonas putida*
Strain	
Source	Own breeding
Sampling site	-
Laboratory culture	Yes
Method of cultivation	Stock and preliminary cultures were kept on nutrient medium in agar slant tubes; stock cultures were incubated at 25 °C for 24 h and then washed with sterile saline
Preparation of inoculum for exposure	After incubation the cultures were washed and the extinction was adjusted to 10 corresponding to Formazin standard suspension.
Pretreatment	No adaptation
Initial cell concentration	No data

3.4.3 Test system

Criteria	**Details**
Culturing apparatus	Erlenmeyer flasks (volume: 300 mL)
Number of culture flasks/concentration	2
Aeration device	No data
Measuring equipment	Measurement of turbidity via UV/VIS measurement
Test performed in closed vessels due to significant volatility of TS	Yes. Test vessels stoppered with cotton-lined plastic caps.

3.4.4 Test conditions

Criteria	**Details**	
Test temperature	25 °C	
pH	7	
Aeration of dilution water	No data	

x

	Suspended solids concentration	-
3.4.5	Duration of the test	16 h
3.4.6	Test parameter	Cell multiplication inhibition
3.4.7	Analytical parameter	Turbidity of bacterial suspension
3.4.8	Sampling	At the end of the test
3.4.9	Monitoring of TS concentration	No
3.4.10	Controls	No data
3.4.11	Statistics	Graphical analysis
	4 RESULTS	
4.1	**Preliminary test**	No data
4.1.1	Concentration	-
4.1.2	Effect data	-
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	No data
4.2.2	Actual concentrations of test substance	No analytical monitoring
4.2.3	Growth curves	Not available
4.2.4	Cell concentration data	No data
4.2.5	Concentration/ response curve	Not available
4.2.6	Effect data	16 h EC_3 (Toxicity Threshold = TT) = 1050 mg/L (nominal)
4.2.7	Other observed effects	No data
4.3	**Results of controls**	No data
4.4	**Test with reference substance**	No
4.4.1	Concentrations	-
4.4.2	Results	-
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The cell multiplication inhibition test according to Bringmann & Kühn is decribed using *Pseudomonas putida*. Four-parallel dilution series in Erlenmeyer flasks stoppered with cotton-lined plastic caps were

Section A7.4.1.4/01 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

		prepared and the toxicological effect of 2-propanol on cell multiplication investigated. After 16 h of incubation at 25°C the EC_3 described as Toxicity Threshold (= TT) was determined graphically based on measurement of turbidity. Analytical monitoring of test substance concentration was not performed.
5.2	**Results and discussion**	In the test a 16 h EC_3 (TT) = 1050 mg/L (nominal) was determined. The test is well described and meets generally accepted scientific principles. No information on conentration-response relationship available. Propan-2-ol shows a moderate volatility from aqueous solution (**cf. Doc III A3.2.1**). The test vessels were stoppered with cotton-lined plastic caps. Therefore significant losses due to volatilisation are not to be expected. However, analytical monitoring of test substance concentration was not performed.
5.2.1	EC_{20}	16 h EC_3 = 1050 mg/L (nominal)
5.2.2	EC_{50}	
5.2.3	EC_{80}	
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	2008/07/04
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 A7.4.1.4/02 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

Official use only

1 REFERENCE

1.1	**Reference**	Gerike P, Gode P (1990) The biodegradability and inhibitory threshold concentration of some disinfectants. Chemosphere 21(6), 799-812 (published)
1.2	**Data protection**	No
1.2.1	Data owner	-
1.2.2	Criteria for data protection	-

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes. The study was conducted according to Iso 8192 'Test for inhibition of oxygen consumption by activated sludge' which is comparable to OECD 209.
2.2	**GLP**	[redacted]
2.3	**Deviations**	Yes. *Pseudomonas putida* were used instead of activated sludge.

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	No data
3.1.4	Composition of Product	Not applicable
3.1.5	Further relevant properties	-
3.1.6	Method of analysis	No data
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 IIIA3).
3.3	**Reference substance**	No data
3.3.1	Method of analysis for reference substance	-
3.4	**Testing procedure**	
3.4.1	Culture medium	No information provided.
3.4.2	Inoculum / test organism	

Criteria	Details
Nature	Bacteria
Species	*Pseudomonas putida*
Strain	

Section A7.4.1.4/02 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

		Source	No data
		Sampling site	No data
		Laboratory culture	No data
		Method of cultivation	No data
		Preparation of inoculum for exposure	No data
		Pretreatment	No data
		Initial cell concentration	No data
3.4.3	Test system	**Criteria**	**Details**
		Culturing apparatus	No data
		Number of culture flasks/concentration	No data
		Aeration device	No data
		Measuring equipment	No data
		Test performed in closed vessels due to significant volatility of TS	No data
3.4.4	Test conditions	**Criteria**	**Details**
		Test temperature	No data
		pH	No data
		Aeration of dilution water	No data
		Suspended solids concentration	No data
3.4.5	Duration of the test	No data. According to guideline a contact time of 30 min or 3 hours are recommended.	
3.4.6	Test parameter	Oxygen consumption	
3.4.7	Analytical parameter	Measurement of oxygen	
3.4.8	Sampling	No data	
3.4.9	Monitoring of TS concentration	No data	
3.4.10	Controls	No data	
3.4.11	Statistics	No data	
		4 RESULTS	
4.1	**Preliminary test**	No data	
4.1.1	Concentration	-	
4.1.2	Effect data	-	
4.2	**Results test**		

	substance	
4.2.1	Initial concentrations of test substance	No data
4.2.2	Actual concentrations of test substance	No data
4.2.3	Growth curves	No data
4.2.4	Cell concentration data	No data
4.2.5	Concentration/ response curve	No data
4.2.6	Effect data	EC_0 >1000 mg/L (nominal)
4.2.7	Other observed 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 Iso 8192 'Test for inhibition of oxygen consumption by activated sludge' comparable to OECD 209. *Pseudomonas putida* were used instead of activated sludge. Further details were not reported in the publication.
5.2	**Results and discussion**	In the guideline test investigating the inhibition of oxygen consumption by *Pseudomonas putida* an EC_0 >1000 mg/L (test duration not stated) was observed. Further details were not reported. As the test was conducted according to guideline the study is regarded as valid.
5.2.1	EC_0	>1000 mg/L (nominal)
5.2.2	EC_{50}	-
5.2.3	EC_{80}	-
5.3	**Conclusion**	[redacted]
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	[redacted]

Section A7.4.1.4/02 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2008/07/03
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 A7.4.1.4/03 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	Klecka GM, Landi LP, Rodner KM (1985) Evaluation of the OECD Activated Sludge, Respiration Inhibition Test. Chemosphere 14, 1239-1251 (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 209 'Activated sludge, respiration inhibition test' (1981)	
2.2	**GLP**	[redacted]	
2.3	**Deviations**	Yes. The synthetic sewage stock solution was prepared with increased level of K_2HPO_4 (28 g instead of 2.8 g).	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Propan-2-ol	x
3.1.1	Lot/Batch number	-	
3.1.2	Specification	i-propanol	
3.1.3	Purity	Reagent grade	
3.1.4	Composition of Product	Not applicable	
3.1.5	Further relevant properties	-	
3.1.6	Method of analysis	Analytically monitoring of TS not 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 2-propanol is moderately volatile from aqueous solution (**cf. Doc III A3.2.1**). Stock solutions of the test chemical were prepared in deionised water. The stock solutions (0.5 to 5 g/L) were adjusted to pH 7.5 ± 0.5.	
3.3	**Reference substance**	Yes. 3,5-Dichlorophenol as recommended in the guideline.	
3.3.1	Method of analysis for reference substance	No information	
3.4	**Testing procedure**		
3.4.1	Culture medium	Synthetic sewage feed (composition per liter of activated sludge)	x

Bacto-Peptone	16 g
Bacto-Beef extract	11 g
urea	3 g
K_2HPO_4	28 g

Section A7.4.1.4/03 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

$MgSO_4$ x 7 H_2O	0.2 g
$CaCl_2$ x $2H_2O$	0.4 g
NaCl	0.7 g

Final pH of the stock solution was adjusted to pH 7.0 with H_3PO_4.

3.4.2 Inoculum / test organism

Criteria	**Details**
Nature	Activated sludge
Species	-
Strain	-
Source	Activated sludge from a local municipal wastewater treatment plant
Sampling site	municipal wastewater treatment plant
Laboratory culture	No
Method of cultivation	-
Preparation of inoculum for exposure	Stock solution adjusted to pH 7.5 ± 0.5; preparation of test reaction mixtures: addition of 16 mL of synthetic sewage stock solution and the desired amount of the test chemical to 500 mL graduated cylinder; dilution with deionised water to 300 mL; addition of activated sludge (200 mL; ca. 800 mg of suspended solids dw); contents (final volume of 500 mL) transferred to a 1 liter bottle; bottle aerated at a a rate of 0.5 and 1 L/min for 3 h at 21 °C
Pretreatment	No adaptation
Initial cell concentration	400 mg suspended solids/L

3.4.3 Test system

Criteria	**Details**
Culturing apparatus	1 L bottle
Number of culture flasks/concentration	No information
Aeration device	Pasteur pipette
Measuring equipment	Polarographic oxygen electrode and an ionanalyzer
Test performed in closed vessels due to significant volatility of TS	No

3.4.4 Test conditions

Criteria	**Details**
Test temperature	21 °C
pH	Initial: 7.5 ± 0.5

Section A7.4.1.4/03 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

	Aeration of dilution water	Yes. 0.5 and 1 L/min
	Suspended solids concentration	400 mg/L

3.4.5	Duration of the test	3 h
3.4.6	Test parameter	respiration inhibition
3.4.7	Analytical parameter	oxygen consumption
3.4.8	Sampling	At the end of the test
3.4.9	Monitoring of TS concentration	No analytical monitoring
3.4.10	Controls	Controls (test substance omitted) were prepared at the beginning and end of the study (no further information provided)
3.4.11	Statistics	In respect to propan-2-ol only graphic data analysis was performed. a. Thompson's method of moving averages (Thompson WR (1947) Use of moving averages and interpolation to estimate median-effective dose. Bact Rev 11, 115) and b. probit-transformation model (Larson RJ, Schaeffer SL (1982) A rapid method for determining the toxicity of chemicals to activated sludge. Water Res 16, 675) were not used for data analysis
		4 RESULTS
4.1	**Preliminary test**	No information
4.1.1	Concentration	-
4.1.2	Effect data	-
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 of test substance concentration
4.2.3	Growth curves	No information
4.2.4	Cell concentration data	400 mg suspended solids/L
4.2.5	Concentration/ response curve	Not available
4.2.6	Effect data	3 h-EC_{50} >1000 mg/L
4.2.7	Other observed effects	No
4.3	**Results of controls**	No information in respect to controls (test substance omitted) reported.
4.4	**Test with**	Performed

Section A7.4.1.4/03 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

	reference substance	
4.4.1	Concentrations	No information
4.4.2	Results	3 h EC_{50} = 12.2 - 12.5 mg/L (3,5-dichlorophenol)
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The study was conducted according to OECD guideline 209 'Activated sludge, respiration inhibition test' (1981). Deviation from guideline: The synthetic sewage stock solution was prepared with increased level of K_2HPO_4 (28 g instead of 2.8 mg).
5.2	**Results and discussion**	As result of the test a 3 h EC_{50} >1000 mg/L was determined. The activity of the inoculum was checked using 3,5-dichlorophenol: The results were within the ranges given in the guideline (3 h EC_{50} range: 5-30 mg/L). The guideline study is well documented and the validity criteria are fulfilled.
5.2.1	EC_{20}	-
5.2.2	EC_{50}	3 h EC_{50} >1000 mg/L
5.2.3	EC_{80}	-
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	2008/07/03
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Section A7.4.1.4/03 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

	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 A7.4.2 Annex Point IIA 7.5	Bioconcentration, aquatic	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	Technically not feasible [ ] Scientifically unjustified [X]	
Limited exposure [ ]	Other justification [ ]	
Detailed justification:	[redacted]	
Reference:	[redacted]	

Section A7.4.2 Annex Point IIA 7.5	Bioconcentration, aquatic
	[redacted]
Undertaking of intended data submission [ ]	[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	2008/07/04
Evaluation of applicant's justification	[redacted]
Conclusion	[redacted]
Remarks	[redacted]
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.4.3.1 Prolonged toxicity to fish

Annex Point IIIA XIII.2.1 *Oryzias latipes*

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted] (1998) Final Report, Prolonged Toxicity Test on Oryzias latipes to 2-Propanol. [redacted] [redacted] 2007 Chemical Risk Information Platform (CHRIP) Total Search System for Chemical Substances: 2-Propanol; [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**	Yes. [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]
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**	Yes: copper sulfate pentahydrate
3.3.1	Method of analysis for reference	No data

Section A7.4.3.1 Prolonged toxicity to fish

Annex Point IIIA XIII.2.1 *Oryzias latipes*

substance

3.4 Testing procedure

3.4.1	Dilution water	[redacted]
3.4.2	Test organisms	[redacted]
3.4.3	Test system	[redacted]

Section A7.4.3.1 Prolonged toxicity to fish

Annex Point IIIA XIII.2.1 *Oryzias latipes*

		[redacted]
3.4.4	Test conditions	[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**	Not performed
4.1.1	Concentration	Not applicable
4.1.2	Number/ percentage of animals showing adverse effects	Not applicable
4.1.3	Nature of adverse effects	Not applicable
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	[redacted]

Section A7.4.3.1 Prolonged toxicity to fish

Annex Point IIIA XIII.2.1 *Oryzias latipes*

4.2.2	Actual concentrations of test substance	[redacted]
4.2.3	Effect data (Mortality)	[redacted]
4.2.4	Concentration / response curve	[redacted]
4.2.5	Other effects	[redacted]
4.3	**Results of controls**	[redacted]
4.3.1	Number/ percentage of animals showing adverse effects	[redacted]
4.3.2	Nature of adverse effects	[redacted]
4.4	**Test with reference substance**	Copper sulfate pentahydrate
4.4.1	Concentrations	No data
4.4.2	Results	96 h LC_{50} = 0.427 mg/L

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	[redacted]
5.2	**Results and discussion**	[redacted]

Section A7.4.3.1 Prolonged toxicity to fish

Annex Point IIIA XIII.2.1 *Oryzias latipes*

[redacted]

5.2.1	LC_0	[redacted]
5.2.2	LC_{50}	[redacted]
5.2.3	LC_{100}	[redacted]
5.3	**Conclusion**	[redacted]
5.3.1	Other Conclusions	
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	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 A7.4.3.2 Annex Point IIIA XIII 2.2	Effects on reproduction and growth rate of fish	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] Limited exposure [ ]	Technically not feasible [ ] Scientifically unjustified [X] Other justification [ ]	
Detailed justification: References:	[redacted] [redacted] x [redacted] x [redacted] [redacted] x [redacted] [redacted] [redacted]	
Undertaking of intended data submission [ ]	[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	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	

Section A7.4.3.2 Annex Point IIIA XIII 2.2	Effects on reproduction and growth rate of fish
Remarks	[redacted]
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	