

Section A7.4.1.3/02 Growth inhibition test on algae

Annex Point IIA7.3

5.3.2 Deficiencies



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	<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>

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 wasser-gefährdender 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-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	<i>Microcystis aeruginosa</i> = 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

3.4.4	Test conditions	Test performed in closed vessels due to significant volatility of TS	Yes. The test was conducted in culture tubes stoppered with metal caps
		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 ₆ C 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 EC₃ described 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_bC₃ = 1000 mg/L).

	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

For *Microcystis aeruginosa* an 8 d E_bC₃ = 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 accepted 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_bC₃: 1000 mg/L (nominal)

5.2.2 E₁₅₀ -

5.2.3 E_bC₅₀ -

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	
<i>Use separate "evaluation boxes" to provide transparency as to the comments and views submitted</i>	
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	<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 A7.4.1.3/04 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, completely 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 described 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	<i>Pseudokirchneriella subcapitata</i> (former scientific name: <i>Selenastrum</i>)

Section A7.4.1.3/04 Growth inhibition test on algae

Annex Point IIA7.3

		<i>capricornutum</i>)														
	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	<table border="1"> <thead> <tr> <th>Criteria</th> <th>Details</th> </tr> </thead> <tbody> <tr> <td>Volume of culture flasks</td> <td>300 mL</td> </tr> <tr> <td>Culturing apparatus</td> <td>BOD bottles placed on orbital shaker at 100 rpm</td> </tr> <tr> <td>Light quality</td> <td>Light intensity: 65 µE m⁻² s⁻¹ (±10%)</td> </tr> <tr> <td>Procedure for suspending algae</td> <td>orbital shaker at 100 rpm</td> </tr> <tr> <td>Number of vessels/ concentration</td> <td>3 replicates</td> </tr> <tr> <td>Test performed in closed vessels due to significant volatility of TS</td> <td>Yes. BOD bottles</td> </tr> </tbody> </table>	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 bottles
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 bottles															
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>24 ± 1 °C</td> </tr> <tr> <td>pH</td> <td>No information</td> </tr> <tr> <td>Aeration of dilution water</td> <td>No, dilution water was stripped by nitrogen gas to reduce dissolved oxygen level.</td> </tr> <tr> <td>Light intensity</td> <td>65 µE m⁻² s⁻¹ (±10%)</td> </tr> <tr> <td>Photoperiod</td> <td>No information (14 h light/10 h dark, according to guideline)</td> </tr> </tbody> </table>	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)		
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 _μ 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 <i>Pseudokirchmeriella subcapitata</i> in the cell multiplication inhibition test (48 h E _μ C ₅₀ = 10500 mg/L).	X

	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

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 completely.	
5.2.1	NOEC	Not reported	
5.2.2	E _r C ₅₀	10500 mg/L (48 h)	
5.2.3	E _b C ₅₀		
5.3	Conclusion	[REDACTED]	x
5.3.1	Reliability	[REDACTED]	
5.3.2	Deficiencies	[REDACTED]	

Evaluation by Competent Authorities	
<i>Use separate "evaluation boxes" to provide transparency as to the comments and views submitted</i>	
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	<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 A7.4.1.3/05 Growth inhibition test on algae
Annex Point II A7.3

JUSTIFICATION FOR NON-SUBMISSION OF DATA

Official
use only

Other existing data Technically not feasible Scientifically unjustified

Limited exposure Other justification

Detailed justification:

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

References:

[REDACTED]

[REDACTED]

Section A7.4.1.3/05 Growth inhibition test on algae

Annex Point II A7.3

	[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 Growth inhibition test on algae

Annex Point IIA7.3

Evaluation of applicant's justification

[Redacted text block]

Conclusion

[Redacted text block]

Remarks

[Redacted text block]

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

Section A7.4.1.3/06 Growth inhibition test on algae

Annex Point IIA7.3

Official
use only

		1 REFERENCE				
1.1 Reference		<p>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 <i>Selenastrum capricornutum</i>. Ecotoxicol. Environ. Safety 71, 166-171 (published)</p> <p>██████████ (2013) Estimation of the EC10 value from the algal test published by Cho et al. 2008 ██████████</p>				
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		<p>Yes</p> <p>OECD guideline 201 (“freshwater alga and cyanobacteria, Growth Inhibition” (2002)</p> <p>OPPTS 850.5400 “Algal Toxicity, Tiers I and II”</p>				
2.2 GLP		██████████				
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		<table border="1" style="width: 100%;"> <thead> <tr> <th style="width: 50%;">Criteria</th> <th style="width: 50%;">Details</th> </tr> </thead> <tbody> <tr> <td> </td> <td> </td> </tr> </tbody> </table>	Criteria	Details		
Criteria	Details					

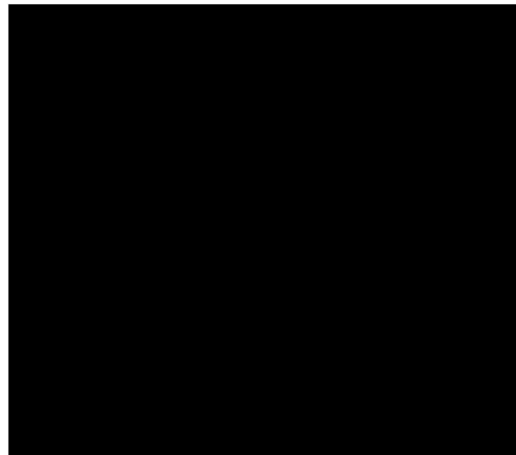
Section A7.4.1.3/06 Growth inhibition test on algae

Annex Point IIA7.3

	Species	<i>Selenastrum capricornutum</i>														
	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 $30 \pm 5 \mu\text{E m}^{-2} \text{s}^{-1}$, using warm-white fluorescent located on top of the shaker. All the flasks were maintained in the shaker incubator at $25 \pm 5 \text{ }^\circ\text{C}$ for 7 days.														
	Pretreatment	None														
	Initial cell concentration	No data														
3.4.3	Test system	<table border="1"> <thead> <tr> <th>Criteria</th> <th>Details</th> </tr> </thead> <tbody> <tr> <td>Volume of culture flasks</td> <td>250 ml Erlenmeyer flasks, fill volume 60 mL</td> </tr> <tr> <td>Culturing apparatus</td> <td>Shaker incubator at 170 rpm</td> </tr> <tr> <td>Light quality</td> <td>Warm-white fluorescent tubes</td> </tr> <tr> <td>Procedure for suspending algae</td> <td>Shaking</td> </tr> <tr> <td>Number of vessels/ concentration</td> <td>2 (test substance) 3 (control)</td> </tr> <tr> <td>Test performed in closed vessels due to significant volatility of TS</td> <td>No data</td> </tr> </tbody> </table>	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
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	<table border="1"> <thead> <tr> <th>Criteria</th> <th>Details</th> </tr> </thead> <tbody> <tr> <td>Test temperature</td> <td>25 °C</td> </tr> <tr> <td>pH</td> <td>No data</td> </tr> <tr> <td>Aeration of dilution water</td> <td>No</td> </tr> <tr> <td>Light intensity</td> <td>$30 \pm 5 \mu\text{E m}^{-2} \text{s}^{-1}$</td> </tr> <tr> <td>Photoperiod</td> <td>Continuous illumination</td> </tr> </tbody> </table>	Criteria	Details	Test temperature	25 °C	pH	No data	Aeration of dilution water	No	Light intensity	$30 \pm 5 \mu\text{E m}^{-2} \text{s}^{-1}$	Photoperiod	Continuous illumination		
Criteria	Details															
Test temperature	25 °C															
pH	No data															
Aeration of dilution water	No															
Light intensity	$30 \pm 5 \mu\text{E m}^{-2} \text{s}^{-1}$															
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



4.2.5 Cell concentration data No data

4.2.6 Effect data (cell multiplication inhibition)

Data published:



x

4.2.7 Other observed effects No data

4.3 Results of controls Not applicable, as no reference substance was tested

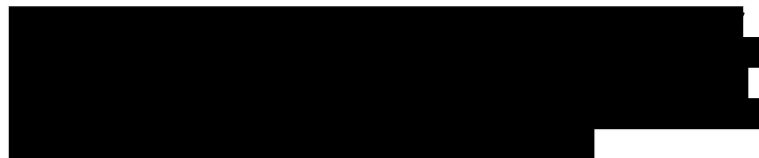
4.4 Test with reference substance Not performed

4.4.1 Concentrations Not applicable

4.4.2 Results Not applicable

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods



5.2 Results and discussion



--	--	--

Section A7.4.1.3/06 Growth inhibition test on algae

Annex Point IIA7.3

		[Redacted]	[Redacted]
		[Redacted]	[Redacted]
5.2.1	NOE _r C	No data, [Redacted]	
5.2.2	E _{r50}	[Redacted]	
5.2.3	E _b C 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	<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 A7.4.1.4/01 **Inhibition to microbial activity (aquatic)**

Annex Point IIA7.4

		1 REFERENCE	Official use only
1.1	Reference	<p>Bringmann G, Kuehn R (1977) Grenzwerte der Schadwirkung wassergefährdender Bakterien (<i>Pseudomonas putida</i>) und Grünalgen (<i>Scenedesmus quadricauda</i>) im Zellvermehrungshemmtest. Z Wasser Abwasser-Forschung 10, 87-98 (published)</p> <p>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)</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	No. No guidelines available at the time the study was conducted.	
2.2	GLP		
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 ₂ HPO ₄ , anhydrous	0.6 g
KH ₂ PO ₄	0.3 g
MgSO ₄ x 7 H ₂ O	0.2 g
Glucose	10 g
Difco Bacto agar	18 g
FeSO ₄ x 7 H ₂ O	0.01 g
Trace elements solution	1.5 mL

Trace elements (in g per liter)

Al ₂ (SO ₄) x 18 H ₂ O	0.055
KJ	0.028
KBr	0.028
TiO ₂	0.055
SnCl ₂ x 2 H ₂ O	0.028
LiCl	0.028
MnCl ₂ x 4 H ₂ O	0.389
H ₃ BO ₃	0.614
ZnSO ₄ x 7 H ₂ O	0.055
CuSO ₄ x 5 H ₂ O	0.055
NiSO ₄ x 6 H ₂ O	0.059
Co(NO ₃) ₂ x 6 H ₂ O	0.055

Vitamine

D-Biotin	0.2 mg
Nicotinic acid	2 mg
Thiamine HCl	1 mg
p-aminobenzoic acid	1 mg
D-Panhotenic 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
NaNO ₃	4.24 g

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

Annex Point IIA7.4

K ₂ HPO ₄ , anhydrous	2.4 g
KH ₂ PO ₄	1.2 g
Trace elements solution	30 mL

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

FeSO ₄ x 7 H ₂ O	0.2 g
MgSO ₄ x 7 H ₂ O	4 g

3.4.2 Inoculum /
test organism

Criteria	Details
Nature	Bacteria
Species	<i>Pseudomonas putida</i>
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

Section A7.4.1.4/01 Inhibition to microbial activity (aquatic)**Annex Point IIA7.4**

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 EC₃ (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 described 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

5.2 Results and discussion

prepared and the toxicological effect of 2-propanol on cell multiplication investigated. After 16 h of incubation at 25°C the EC₃ described as Toxicity Threshold (= TT) was determined graphically based on measurement of turbidity. Analytical monitoring of test substance concentration was not performed.

In the test a 16 h EC₃ (TT) = 1050 mg/L (nominal) was determined. The test is well described and meets generally accepted scientific principles. No information on concentration-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 EC₂₀

16 h EC₃ = 1050 mg/L (nominal)

5.2.2 EC₅₀

5.2.3 EC₈₀

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	<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 A7.4.1.4/02 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 XXXXXXXXXX

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	<i>Pseudomonas putida</i>
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	<table border="1"> <thead> <tr> <th>Criteria</th> <th>Details</th> </tr> </thead> <tbody> <tr> <td>Culturing apparatus</td> <td>No data</td> </tr> <tr> <td>Number of culture flasks/concentration</td> <td>No data</td> </tr> <tr> <td>Aeration device</td> <td>No data</td> </tr> <tr> <td>Measuring equipment</td> <td>No data</td> </tr> <tr> <td>Test performed in closed vessels due to significant volatility of TS</td> <td>No data</td> </tr> </tbody> </table>	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
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	<table border="1"> <thead> <tr> <th>Criteria</th> <th>Details</th> </tr> </thead> <tbody> <tr> <td>Test temperature</td> <td>No data</td> </tr> <tr> <td>pH</td> <td>No data</td> </tr> <tr> <td>Aeration of dilution water</td> <td>No data</td> </tr> <tr> <td>Suspended solids concentration</td> <td>No data</td> </tr> </tbody> </table>	Criteria	Details	Test temperature	No data	pH	No data	Aeration of dilution water	No data	Suspended solids concentration	No data		
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	

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

Annex Point IIA7.4

	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. <i>Pseudomonas putida</i> 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 <i>Pseudomonas putida</i> 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	<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 A7.4.1.4/03 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

		1 REFERENCE	Official use only								
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	██████████									
2.3	Deviations	Yes. The synthetic sewage stock solution was prepared with increased level of K ₂ HPO 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								
		<table border="1"> <tbody> <tr> <td>Bacto-Peptide</td> <td>16 g</td> </tr> <tr> <td>Bacto-Beef extract</td> <td>11 g</td> </tr> <tr> <td>urea</td> <td>3 g</td> </tr> <tr> <td>K₂HPO₄</td> <td>28 g</td> </tr> </tbody> </table>	Bacto-Peptide	16 g	Bacto-Beef extract	11 g	urea	3 g	K ₂ HPO ₄	28 g	
Bacto-Peptide	16 g										
Bacto-Beef extract	11 g										
urea	3 g										
K ₂ HPO ₄	28 g										

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

Annex Point IIA7.4

MgSO ₄ × 7 H ₂ O	0.2 g
CaCl ₂ × 2H ₂ O	0.4 g
NaCl	0.7 g

Final pH of the stock solution was adjusted to pH 7.0 with H₃PO₄.

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 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-EC₅₀ >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 ₂ HPO 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	<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 A7.4.2 **Bioconcentration, aquatic**
Annex Point II A 7.5

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]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Reference:

[REDACTED]

Section A7.4.2 Annex Point IIA 7.5	Bioconcentration, aquatic
	[REDACTED]
Undertaking of intended data submission []	Not applicable, no study is planned.
Evaluation by Competent Authorities	
<i>Use separate "evaluation boxes" to provide transparency as to the comments and views submitted</i>	
EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	2008/07/04
Evaluation of applicant's justification	[REDACTED]
Conclusion	[REDACTED]
Remarks	[REDACTED]
COMMENTS FROM OTHER MEMBER STATE <i>(specify)</i>	
Date	<i>Give date of comments submitted</i>
Evaluation of applicant's justification	<i>Discuss if deviating from view of rapporteur member state</i>
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>
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]
[REDACTED] 2007 Chemical Risk Information Platform (CHRIP) Total Search System for Chemical Substances: 2-Propanol; [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** 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]	[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]

3.4.3 Test system

[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[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]
		[Table with redacted cells]
		[Redacted]
		[Redacted]

Section A7.4.3.1 Prolonged toxicity to fish

Annex Point IIIA XIII.2.1 *Oryzias latipes*

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	[REDACTED]	
5.3.2	Reliability	[REDACTED]	
5.3.3	Deficiencies	[REDACTED]	

Evaluation by Competent Authorities	
<i>Use separate "evaluation boxes" to provide transparency as to the comments and views submitted</i>	
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	<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 A7.4.3.2		Effects on reproduction and growth rate of fish	
Annex Point IIIA XIII 2.2			
JUSTIFICATION FOR NON-SUBMISSION OF DATA			Official use only
Other existing data <input checked="" type="checkbox"/>	Technically not feasible <input type="checkbox"/>	Scientifically unjustified <input checked="" type="checkbox"/>	
Limited exposure <input type="checkbox"/>	Other justification <input type="checkbox"/>		
Detailed justification:	<div style="background-color: black; width: 100%; height: 15px; margin-bottom: 5px;"></div> <div style="background-color: black; width: 100%; height: 15px; margin-bottom: 5px;"></div> <div style="background-color: black; width: 100%; height: 15px; margin-bottom: 5px;"></div> <div style="background-color: black; width: 100%; height: 15px; margin-bottom: 5px;"></div> <div style="background-color: black; width: 100%; height: 15px; margin-bottom: 5px;"></div> <div style="background-color: black; width: 100%; height: 15px; margin-bottom: 5px;"></div>		
References:	<div style="background-color: black; width: 100%; height: 15px;"></div>		
Undertaking of intended data submission <input type="checkbox"/>	<div style="background-color: black; width: 100%; height: 15px;"></div>		
Evaluation by Competent Authorities			
<i>Use separate "evaluation boxes" to provide transparency as to the comments and views submitted</i>			
EVALUATION BY RAPPORTEUR MEMBER STATE			
Date	2008/06/30		
Evaluation of applicant's justification	<div style="background-color: black; width: 100%; height: 15px;"></div>		
Conclusion	<div style="background-color: black; width: 100%; height: 15px;"></div>		

Section A7.4.3.2 **Effects on reproduction and growth rate of fish**

Annex Point IIIA XIII 2.2

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 7.4.3.4/01 **Effects on reproduction and growth rate with an invertebrate species**
Annex Point IIIA XIII 2.4

Daphnia magna STRAUS

offspring	[REDACTED]	
3.4.4 Test system	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]
3.4.5 Test conditions	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]
3.4.6 Duration of the test	[REDACTED]	
3.4.7 Test parameter	[REDACTED]	
3.4.8 Examination / Sampling	[REDACTED]	
3.4.9 Monitoring of TS concentration	[REDACTED]	
3.4.10 Statistics	[REDACTED]	

4 RESULTS

- 4.1 Range finding test** Not performed
- 4.1.1 Concentrations Not applicable

Section 7.4.3.4/01 **Effects on reproduction and growth rate with an invertebrate species**
Annex Point IIIA XIII 2.4

Daphnia magna STRAUS

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]	x
4.2.2	Actual concentrations of test substance	[REDACTED]	
4.2.3	Effect data	[REDACTED]	x
4.2.4	Concentration / response curve	[REDACTED]	
4.2.5	Other effects	[REDACTED]	
4.3	Results of controls	[REDACTED]	x
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]	

Section 7.4.3.4/01 **Effects on reproduction and growth rate with an invertebrate species**
Annex Point IIIA XIII 2.4


Daphnia magna STRAUS

5.2	Results and discussion		x
5.2.1	NOEC		
5.2.2	LOEC		
5.2.3	EC ₃₀ (EC _x)		
5.3	Conclusion		
5.3.1	Reliability		
5.3.2	Deficiencies		

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	
Results and discussion	
Conclusion	
Reliability	
Acceptability	

Section 7.4.3.4/01 **Effects on reproduction and growth rate with an**
Annex Point IIIA XIII 2.4 **invertebrate species**

Daphnia magna STRAUS

Remarks	
Date	COMMENTS FROM ... (<i>specify</i>) <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 7.4.3.4/02 **Effects on reproduction and growth rate with an**
Annex Point IIIA XIII 2.4 **invertebrate species**

Daphnia magna STRAUS

3.4.1 Dilution water

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

Criteria	Details
Species	<i>Daphnia magna</i>
Strain / Clone	Not stated
Source	No data
Age	<1 d
Breeding method	No data
Kind of food	<i>Chlorella spec.</i>
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 (aim of the study was to examine the growth of the daphnids)

3.4.4 Test system

Criteria	Details
Test type	Semi-static
Renewal of test solution	renewing rate: 3 times a week (no further information)
Volume of test vessels	1 L
Volume/animal	67 mL/animal

Section 7.4.3.4/02 **Effects on reproduction and growth rate with an invertebrate species**
Annex Point IIIA XIII 2.4

Daphnia magna STRAUS

3.4.5	Test conditions	Number of animals/vessel	15
		Number of vessels/ concentration	2
		Test performed in closed vessels due to significant volatility of TS	No data
		Criteria	Details
		Test temperature	19 ± 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 16 d
- 3.4.7 Test parameter Growth (measured as length)
- 3.4.8 Examination / Sampling At the start of the experiment and after 16 days the length of the daphnids was measured.
- 3.4.9 Monitoring of TS concentration Yes. Just before and after renewal of the test solution the actual concentrations were determined
- 3.4.10 Statistics Student's t-test

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 Just before and after renewal of the test solution the actual concentrations were determined (no further information)
 - 4.2.3 Effect data 16 d NOEC_{Growth} = 141 mg/L (at p < 0.001).

Section 7.4.3.4/02 **Effects on reproduction and growth rate with an**
Annex Point IIIA XIII 2.4 **invertebrate species**

Daphnia magna STRAUS

		No further information
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 6502 (1980) which is comparable to OECD guideline 202.

In deviation of the standard procedure the test duration was 16 d.

The test was conducted in 1 L flasks and 15 daphnids (tests conducted in duplicate: 30 daphnids per concentration). The test solution was renewed 3 times a week and the actual concentration of the test substance measured analytically. The decrease of the concentration till renewing the solutions was maximally 20%. At the start of the experiment and after 16 days the length of the daphnids was measured. The NOEC was tested with Student's t-test at $p < 0.001$.

5.2 Results and discussion In the chronic study with *Daphnia magna* a 16 d $NOEC_{Growth} = 141$ mg/L was determined in respect to growth (endpoint length). Mortality or reproduction was not subject of the study. No information is provided about the mortality of parent animals at test termination.

As the study was conducted according to a national standard method with an acceptable modification (test duration: 16 d) and the information provided the study can be regarded as valid.

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. The analytical monitoring of test substance concentrations showed that the actual measured concentrations were in all cases $\geq 80\%$ of initially applied.

5.2.1 NOEC 16 d $NOEC_{Growth} = 141$ mg/L

5.2.2 LOEC -

5.2.3 EC_{50} (EC_x) -

5.3 Conclusion

[REDACTED]

5.3.1 Reliability [REDACTED]

5.3.2 Deficiencies [REDACTED]

[REDACTED]

Section 7.4.3.4/02
Annex Point IIIA XIII 2.4

Effects on reproduction and growth rate with an invertebrate species

Daphnia magna STRAUS

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 ... (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 7.4.3.4/03 **Effects on reproduction and growth rate with an**
Annex Point IIIA XIII 2.4 **invertebrate species**

Official
use only

- 1 REFERENCE**
- 1.1 Reference** [REDACTED]
- 1.2 Data protection** Yes
- 1.2.1 Data owner Huels AG
- 1.2.2 Criteria for data protection Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

- 2.1 Guideline study** No. [REDACTED]
- 2.2 GLP** [REDACTED]
- 2.3 Deviations** No data

3 METHOD

- 3.1 Test material** Propan-2-ol
- 3.1.1 Lot/Batch number No information
- 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 No information
- 3.1.6 Method of analysis No data
- 3.2 Preparation of TS solution for poorly soluble or volatile test substances** No data
- 3.3 Reference substance** No data
- 3.3.1 Method of analysis for reference substance Not applicable

3.4 Testing procedure

3.4.1 Dilution water

Criteria	Details
Source	No data
Salinity	No data
Hardness	No data
pH	No data
Ca / Mg ratio	No data

**Section 7.4.3.4/03 Effects on reproduction and growth rate with an
Annex Point IIIA XIII 2.4 invertebrate species**

3.4.2 Test organisms

Na / K ratio	No data
Oxygen content	No data
Conductance	No data
TOC	No data
Holding water different from dilution water	No data
Criteria	Details
Strain / Clone	No data
Source	No data
Age	No data
Breeding method	No data
Kind of food	No data
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

No data

3.4.4 Test system

Criteria	Details
Test type	No data
Renewal of test solution	No data
Volume of test vessels	No data
Volume/animal	No data
Number of animals/vessel	No data
Number of vessels/ concentration	No data
Test performed in closed vessels due to significant volatility of TS	No data

3.4.5 Test conditions

Criteria	Details
Test temperature	No data
Dissolved oxygen	No data
pH	No data
Adjustment of pH	No data
Aeration of dilution water	No data
Quality/Intensity of	No data

Section 7.4.3.4/03 **Effects on reproduction and growth rate with an**
Annex Point IIIA XIII 2.4 **invertebrate species**

irradiation	
Photoperiod	No data

- 3.4.6 Duration of the test 21 days
- 3.4.7 Test parameter No data
- 3.4.8 Examination /
Sampling No data
- 3.4.9 Monitoring of TS
concentration No
- 3.4.10 Statistics No data

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 Analytical monitoring not performed.
- 4.2.3 Effect data 21 d NOEC = 30 mg/L
21 d EC₂₀ = 100 mg/L
21 d EC₅₀ >100 mg/L (no further information available).
- 4.2.4 Concentration /
response curve No data
- 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** Chronic toxicity towards *Daphnia magna* was tested according to a
proposal of the Federal Environmental Protection Agency 'Prolonged

Section 7.4.3.4/03 **Effects on reproduction and growth rate with an**
Annex Point IIIA XIII 2.4 **invertebrate species**

5.2	Results and discussion	<p>toxicity-test with <i>Daphnia magna</i>. Further information is not available.</p> <p>The chronic toxicity of propan-2-ol towards <i>Daphnia magna</i> was tested according to a proposal of the Federal Environmental Agency.</p> <p>The following results are available: 21 d NOEC=30 mg/L, 21 d EC₂₉=100 mg/L, and 21 d EC₅₀>100 mg/L. No further information is available.</p> <p>The original study is not available and the data given not assignable.</p>
5.2.1	NOEC	21 d NOEC=30 mg/L
5.2.2	LOEC	
5.2.3	EC ₅₀ (EC _x)	21 d EC ₂₉ =100 mg/L, and 21 d EC ₅₀ >100 mg/L.
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	2008/06/30
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>

Section 7.4.3.4/03 **Effects on reproduction and growth rate with an**
Annex Point IIIA XIII 2.4 **invertebrate species**

Acceptability

Discuss if deviating from view of rapporteur member state

Remarks

Section 7.4.3.4/04 **Effects on reproduction and growth rate with an invertebrate species**
Annex Point IIIA XIII 2.4

Daphnia magna STRAUS

3.4.2 Test organisms

	trophic levels. Ecotoxicol Environ Safe 6, 113-128
Salinity	No data
Hardness	Approx. 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
Criteria	Details
Species	<i>Daphnia magna</i>
Strain / Clone	Strain not stated
Source	No data
Age	<1 d
Breeding method	No data
Kind of food	<i>Chlorella spec.</i>
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 (aim of the study was to examine the growth of the daphnids)

3.4.4 Test system

Criteria	Details
Test type	Semi-static
Renewal of test solution	renewing rate: 3 times a week (no further information)
Volume of test vessels	1 L
Volume/animal	67 mL/animal
Number of animals/vessel	15
Number of vessels/ concentration	2
Test performed in closed vessels due to significant	No data

Section 7.4.3.4/04 **Effects on reproduction and growth rate with an invertebrate species**
Annex Point IIIA XIII 2.4*Daphnia magna* STRAUS

		volatility of TS	
3.4.5	Test conditions	Criteria	Details
		Test temperature	19 ± 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	Until control daphnids produced four broods.	x
3.4.7	Test parameter	Reproduction and growth	
3.4.8	Examination / Sampling	At the start and at the end of the experiment the length of the daphnids was measured.	
3.4.9	Monitoring of TS concentration	Yes. Just before and after renewal of the test solution the actual concentrations were determined.	
3.4.10	Statistics	Student's t-test (p < 0.01)	
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	Just before and after renewal of the test solution the actual concentrations were determined. Concentrations were 80 - 104% of calculated amount. Decrease during the test was average 9% (Maximum 26%).	
4.2.3	Effect data	NOEC _{Reproduction} = 1408 mg/L NOEC _{Growth} = 774 mg/L (at p < 0.01). No further information.	
4.2.4	Concentration / response curve	Not available	
4.2.5	Other effects	No data	