

**Section A7.4.1.3/01 Growth inhibition test on algae**

**Annex Point IIA7.3**

*Selenastrum capricornutum*

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

**4 RESULTS**

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

**4.2 Results test substance**

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



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

Section A7.4.1.3/01

Growth inhibition test on algae

Annex Point IIA7.3

*Selenastrum capricornutum*

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

4.2.6 Effect data  
(cell multiplication  
inhibition)

[REDACTED]

4.2.7 Other observed  
effects

[REDACTED]

4.3 Results of controls cf. 4.2.3

4.4 Test with  
reference  
substance Potassium dichromate

4.4.1 Concentrations No data

4.4.2 Results 48 h E<sub>0</sub>C<sub>50</sub> = 0.369 mg/L

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and  
methods

[REDACTED]

5.2 Results and  
discussion

[REDACTED]

x

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

5.2.1 NOE<sub>r</sub>C

[REDACTED]

5.2.2 E<sub>r</sub>C<sub>50</sub>

[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**

		<b>1 REFERENCE</b>
<b>1.1</b>	<b>Reference</b>	Bringmann G, Kuehn R (1977) Grenzwerte der Schadwirkung wassergefährdender Stoffe gegen Bakterien ( <i>Pseudomonas putida</i> ) und Grünalgen ( <i>Scenedesmus quadricauda</i> ) im Zellvermehrungshemmtest. Z Wasser Abwasser-Forsch 10, 87-98 (published) Bringmann G, Kuehn R (1978) Grenzwerte der Schadwirkung wassergefährdender Stoffe gegen Blaualgen ( <i>Microcystis aeruginosa</i> ) und Grünalgen ( <i>Scenedesmus quadricauda</i> ) im Zellvermehrungshemmtest. Vom Wasser 50, 45-60 (published) Bringmann G, Kuehn R (1980) Comparison of the toxicity thresholds of water pollutants to bacteria, algae, and protozoa in the cell multiplication inhibition test. Water Res 14, 231-241 (published)
<b>1.2</b>	<b>Data protection</b>	No
1.2.1	Data owner	-
1.2.2	Criteria for data protection	No data protection claimed
		<b>2 GUIDELINES AND QUALITY ASSURANCE</b>
<b>2.1</b>	<b>Guideline study</b>	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.
<b>2.2</b>	<b>GLP</b>	
<b>2.3</b>	<b>Deviations</b>	-
		<b>3 MATERIALS AND METHODS</b>
<b>3.1</b>	<b>Test material</b>	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
<b>3.2</b>	<b>Preparation of TS solution for poorly soluble or volatile test substances</b>	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
<b>3.3</b>	<b>Reference substance</b>	No information
3.3.1	Method of analysis for reference substance	-

Official  
use only

## Section A7.4.1.3/02 Growth inhibition test on algae

## Annex Point IIA7.3

## 3.4 Testing procedure

## 3.4.1 Culture medium

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

The aforementioned nutrients were dissolved in double-distilled water. 10 mL of trace elements solution was added. Double-distilled water was used to complete the solution to 1 L. The pH was adjusted to pH 7.0 using Na<sub>2</sub>CO<sub>3</sub> solution.

## 3.4.2 Test organisms

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

## 3.4.3 Test system

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

## Section A7.4.1.3/02 Growth inhibition test on algae

## Annex Point IIA7.3

		from each other												
	Procedure for suspending algae	Shaking once a day												
	Number of vessels/ concentration	3 tubes												
	Test performed in closed vessels due to significant volatility of TS	Yes. The test was conducted in culture tubes stoppered with metal caps												
3.4.4	Test conditions	<table border="1"> <thead> <tr> <th>Criteria</th> <th>Details</th> </tr> </thead> <tbody> <tr> <td>Test temperature</td> <td>27 °C</td> </tr> <tr> <td>pH</td> <td>No information</td> </tr> <tr> <td>Aeration of dilution water</td> <td>No information</td> </tr> <tr> <td>Light intensity</td> <td>No information</td> </tr> <tr> <td>Photoperiod</td> <td>Continuous lightning</td> </tr> </tbody> </table>	Criteria	Details	Test temperature	27 °C	pH	No information	Aeration of dilution water	No information	Light intensity	No information	Photoperiod	Continuous lightning
Criteria	Details													
Test temperature	27 °C													
pH	No information													
Aeration of dilution water	No information													
Light intensity	No information													
Photoperiod	Continuous lightning													
3.4.5	Duration of the test	7 or 8 days (according to the literature the EC <sub>3</sub> 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 <sub>3</sub> described as Toxicity Threshold (= TT) determined graphically based on experimental results.												

**4 RESULTS**

<b>4.1</b>	<b>Limit Test</b>	No information
4.1.1	Concentration	-
4.1.2	Number/ percentage of animals showing adverse effects	-
<b>4.2</b>	<b>Results test substance</b>	
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<sub>b</sub>C<sub>3</sub> = 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<sub>3</sub> 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**

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

In a cell multiplication inhibition test according to Bringmann & Kuehn a 7 d/8 d E<sub>b</sub>C<sub>3</sub> = 1800 mg/L (nominal) was determined. In general the study is very well documented. However, it is not stated whether the cultures were in the exponential growth phase during the test period.

2-Propanol shows a very low toxicity towards *Scenedesmus quadricauda* in the cell multiplication inhibition test (7d/8d E<sub>b</sub>C<sub>3</sub>= 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<sub>b</sub>C 7d/8d E<sub>b</sub>C<sub>3</sub>: 1800 mg/L (nominal)
- 5.2.2 E<sub>t50</sub> -
- 5.2.3 E<sub>b</sub>C<sub>50</sub>

**5.3 Conclusion**

[REDACTED]

- 5.3.1 Reliability

■

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

<b>EVALUATION BY RAPPORTEUR MEMBER STATE</b>	
<b>Date</b>	2008/07/04
<b>Materials and Methods</b>	[REDACTED]
<b>Results and discussion</b>	[REDACTED]
<b>Conclusion</b>	[REDACTED]
<b>Reliability</b>	[REDACTED]
<b>Acceptability</b>	[REDACTED]
<b>Remarks</b>	[REDACTED]

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



**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 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 Na<sub>2</sub>CO<sub>3</sub> 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
		<b>Criteria</b>	<b>Details</b>
		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 <sub>3</sub> 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 <sub>3</sub> determined graphically based on experimental results.	

**4 RESULTS**

<b>4.1</b>	<b>Limit Test</b>	No information
4.1.1	Concentration	-
4.1.2	Number/ percentage of animals showing adverse effects	-
<b>4.2</b>	<b>Results test substance</b>	
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 <sub>6</sub> C <sub>3</sub> = 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<sub>3</sub> 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<sub>b</sub>C<sub>3</sub> = 1000 mg/L).

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

For *Microcystis aeruginosa* an 8 d E<sub>b</sub>C<sub>3</sub> = 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<sub>b</sub>C E<sub>b</sub>C<sub>3</sub>: 1000 mg/L (nominal)

5.2.2 E<sub>150</sub> -

5.2.3 E<sub>b</sub>C<sub>50</sub> -

**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

<b>Evaluation by Competent Authorities</b>	
<i>Use separate "evaluation boxes" to provide transparency as to the comments and views submitted</i>	
<b>EVALUATION BY RAPPORTEUR MEMBER STATE</b>	
<b>Date</b>	2008/07/04
<b>Materials and Methods</b>	[REDACTED]
<b>Results and discussion</b>	[REDACTED]
<b>Conclusion</b>	[REDACTED]
<b>Reliability</b>	[REDACTED]
<b>Acceptability</b>	[REDACTED]
<b>Remarks</b>	[REDACTED]
<b>COMMENTS FROM ...</b>	
<b>Date</b>	<i>Give date of comments submitted</i>
<b>Materials and Methods</b>	<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>
<b>Results and discussion</b>	<i>Discuss if deviating from view of rapporteur member state</i>
<b>Conclusion</b>	<i>Discuss if deviating from view of rapporteur member state</i>
<b>Reliability</b>	<i>Discuss if deviating from view of rapporteur member state</i>
<b>Acceptability</b>	<i>Discuss if deviating from view of rapporteur member state</i>
<b>Remarks</b>	

**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** ██████████
- 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  
NaNO<sub>3</sub> : 12.75 mg/L, K<sub>2</sub>HPO<sub>4</sub>: 0.52 mg/L, EDTA: 30 µg/L

3.4.2 Test organisms	<b>Criteria</b>	<b>Details</b>
	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 <sup>-2</sup> s <sup>-1</sup> (±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<sup>-2</sup> s<sup>-1</sup> (±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 <sup>-2</sup> s <sup>-1</sup> (±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 <sup>-2</sup> s <sup>-1</sup> (±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<sup>-2</sup> s<sup>-1</sup> (±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 <sup>-2</sup> s <sup>-1</sup> (±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 <sup>-2</sup> s <sup>-1</sup> (±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 <sub>50</sub> determined by probit analysis														



**Section A7.4.1.3/04 Growth inhibition test on algae**

**Annex Point IIA7.3**

**4 RESULTS**

<b>4.1</b>	<b>Limit Test</b>	No information
4.1.1	Concentration	-
4.1.2	Number/ percentage of animals showing adverse effects	-
<b>4.2</b>	<b>Results test substance</b>	
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
<b>4.3</b>	<b>Results of controls</b>	No information
<b>4.4</b>	<b>Test with reference substance</b>	No information
4.4.1	Concentrations	-
4.4.2	Results	-

**5 APPLICANT'S SUMMARY AND CONCLUSION**

<b>5.1</b>	<b>Materials and methods</b>	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
<b>5.2</b>	<b>Results and discussion</b>	Propan-2-ol shows a very low toxicity towards <i>Pseudokirchmeriella subcapitata</i> in the cell multiplication inhibition test (48 h $E_{\mu}C_{50}$ = 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<sub>r</sub>C<sub>50</sub> 10500 mg/L (48 h)
- 5.2.3 E<sub>b</sub>C<sub>50</sub>

**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]

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

**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]

[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]

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

**Section A7.4.1.3/06 Growth inhibition test on algae**

**Annex Point IIA7.3**

Official  
use only

		<b>1 REFERENCE</b>				
<b>1.1 Reference</b>		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) [REDACTED] (2013) Estimation of the EC10 value from the algal test published by Cho et al. 2008 [REDACTED] [REDACTED]				
<b>1.2 Data protection</b>		No				
1.2.1	Data owner	Data published				
1.2.2	Criteria for data protection	No data protection claimed				
		<b>2 GUIDELINES AND QUALITY ASSURANCE</b>				
<b>2.1 Guideline study</b>		Yes OECD guideline 201 (“freshwater alga and cyanobacteria, Growth Inhibition” (2002) OPPTS 850.5400 “Algal Toxicity, Tiers I and II”				
<b>2.2 GLP</b>		[REDACTED]				
<b>2.3 Deviations</b>		No				
		<b>3 MATERIALS AND METHODS</b>				
<b>3.1 Test material</b>		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				
<b>3.2 Preparation of TS solution for poorly soluble or volatile test substances</b>		Not applicable				
<b>3.3 Reference substance</b>		No				
3.3.1	Method of analysis for reference substance	Not applicable				
<b>3.4 Testing procedure</b>						
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><math>30 \pm 5 \mu\text{E m}^{-2} \text{s}^{-1}</math></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

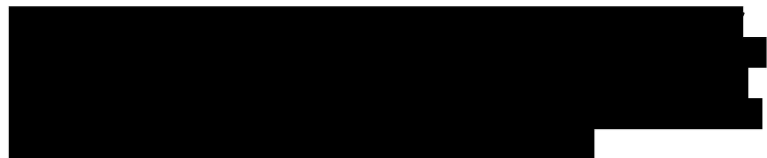
**4.4 Test with reference substance** Not performed

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 <sub>r</sub> C	No data, [Redacted]	
5.2.2	E <sub>r50</sub>	[Redacted]	
5.2.3	E <sub>b</sub> C <sub>50</sub>	No data	
<b>5.3</b>	<b>Conclusion</b>	[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	
<b>EVALUATION BY RAPPORTEUR MEMBER STATE</b>	
<b>Date</b>	2014/02/12
<b>Materials and Methods</b>	[Redacted]
<b>Results and discussion</b>	[Redacted]
<b>Conclusion</b>	[Redacted]
<b>Reliability</b>	[Redacted]
<b>Acceptability</b>	[Redacted]
<b>Remarks</b>	[Redacted]
<b>COMMENTS FROM ...</b>	

**Section A7.4.1.3/06      Growth inhibition test on algae**

**Annex Point IIA7.3**

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



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

**Annex Point IIA7.4**

NaNO <sub>3</sub>	1.06 g
K <sub>2</sub> HPO <sub>4</sub> , anhydrous	0.6 g
KH <sub>2</sub> PO <sub>4</sub>	0.3 g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	0.2 g
Glucose	10 g
Difco Bacto agar	18 g
FeSO <sub>4</sub> x 7 H <sub>2</sub> O	0.01 g
Trace elements solution	1.5 mL

Trace elements (in g per liter)

Al <sub>2</sub> (SO <sub>4</sub> ) x 18 H <sub>2</sub> O	0.055
KJ	0.028
KBr	0.028
TiO <sub>2</sub>	0.055
SnCl <sub>2</sub> x 2 H <sub>2</sub> O	0.028
LiCl	0.028
MnCl <sub>2</sub> x 4 H <sub>2</sub> O	0.389
H <sub>3</sub> BO <sub>3</sub>	0.614
ZnSO <sub>4</sub> x 7 H <sub>2</sub> O	0.055
CuSO <sub>4</sub> x 5 H <sub>2</sub> O	0.055
NiSO <sub>4</sub> x 6 H <sub>2</sub> O	0.059
Co(NO <sub>3</sub> ) <sub>2</sub> x 6 H <sub>2</sub> 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 <sub>12</sub>	2 mg
Double-distilled water	100 mL

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

Glucose	20 g
NaNO <sub>3</sub>	4.24 g

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

## Annex Point IIA7.4

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

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

FeSO <sub>4</sub> x 7 H <sub>2</sub> O	0.2 g
MgSO <sub>4</sub> x 7 H <sub>2</sub> 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**

<b>4.1</b>	<b>Preliminary test</b>	No data
4.1.1	Concentration	-
4.1.2	Effect data	-
<b>4.2</b>	<b>Results test substance</b>	
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 <sub>3</sub> (Toxicity Threshold = TT) = 1050 mg/L (nominal)
4.2.7	Other observed effects	No data
<b>4.3</b>	<b>Results of controls</b>	No data
<b>4.4</b>	<b>Test with reference substance</b>	No
4.4.1	Concentrations	-
4.4.2	Results	-

**5      APPLICANT'S SUMMARY AND CONCLUSION**

<b>5.1</b>	<b>Materials and methods</b>	The cell multiplication inhibition test according to Bringmann & Kühn is described using <i>Pseudomonas putida</i> . 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**

<p><b>5.2 Results and discussion</b></p>	<p>prepared and the toxicological effect of 2-propanol on cell multiplication investigated. After 16 h of incubation at 25°C the EC<sub>3</sub> described as Toxicity Threshold (= TT) was determined graphically based on measurement of turbidity. Analytical monitoring of test substance concentration was not performed.</p> <p>In the test a 16 h EC<sub>3</sub> (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.</p> <p>Propan-2-ol shows a moderate volatility from aqueous solution (<b>cf. Doc III A3.2.1</b>). 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.</p>
<p>5.2.1 EC<sub>20</sub></p>	<p>16 h EC<sub>3</sub> = 1050 mg/L (nominal)</p>
<p>5.2.2 EC<sub>50</sub></p>	
<p>5.2.3 EC<sub>80</sub></p>	
<p><b>5.3 Conclusion</b></p>	<p>[REDACTED]</p>
<p>5.3.1 Reliability</p>	<p>[REDACTED]</p>
<p>5.3.2 Deficiencies</p>	<p>[REDACTED]</p>

<b>Evaluation by Competent Authorities</b>	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
<b>EVALUATION BY RAPPORTEUR MEMBER STATE</b>	
<b>Date</b>	2008/07/04
<b>Materials and Methods</b>	<p>[REDACTED]</p> <p>[REDACTED]</p> <p>[REDACTED]</p> <p>[REDACTED]</p> <p>[REDACTED]</p> <p>[REDACTED]</p> <p>[REDACTED]</p>

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

**Annex Point IIA7.4**

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

**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	<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	
	<b>Criteria</b>	<b>Details</b>
	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	
	<b>Criteria</b>	<b>Details</b>
	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**

<b>4.1</b>	<b>Preliminary test</b>	No data
4.1.1	Concentration	-
4.1.2	Effect data	-
<b>4.2</b>	<b>Results test</b>	

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

**Annex Point IIA7.4**

	<b>substance</b>	
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 <sub>0</sub> >1000 mg/L (nominal)
4.2.7	Other observed effects	No data
<b>4.3</b>	<b>Results of controls</b>	No data
<b>4.4</b>	<b>Test with reference substance</b>	No data
4.4.1	Concentrations	-
4.4.2	Results	-

**5      APPLICANT'S SUMMARY AND CONCLUSION**

<b>5.1</b>	<b>Materials and methods</b>	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.
<b>5.2</b>	<b>Results and discussion</b>	In the guideline test investigating the inhibition of oxygen consumption by <i>Pseudomonas putida</i> an EC <sub>0</sub> >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 <sub>0</sub>	>1000 mg/L (nominal)
5.2.2	EC <sub>50</sub>	-
5.2.3	EC <sub>80</sub>	-
<b>5.3</b>	<b>Conclusion</b>	[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**

<b>Evaluation by Competent Authorities</b>	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
<b>EVALUATION BY RAPPORTEUR MEMBER STATE</b>	
<b>Date</b>	2008/07/03
<b>Materials and Methods</b>	[REDACTED]
<b>Results and discussion</b>	[REDACTED]
<b>Conclusion</b>	[REDACTED]
<b>Reliability</b>	[REDACTED]
<b>Acceptability</b>	[REDACTED]
<b>Remarks</b>	[REDACTED]
<b>COMMENTS FROM ...</b>	
<b>Date</b>	<i>Give date of comments submitted</i>
<b>Materials and Methods</b>	<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>
<b>Results and discussion</b>	<i>Discuss if deviating from view of rapporteur member state</i>
<b>Conclusion</b>	<i>Discuss if deviating from view of rapporteur member state</i>
<b>Reliability</b>	<i>Discuss if deviating from view of rapporteur member state</i>
<b>Acceptability</b>	<i>Discuss if deviating from view of rapporteur member state</i>
<b>Remarks</b>	

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

## Annex Point IIA7.4

		1 REFERENCE	Official use only								
<b>1.1</b>	<b>Reference</b>	Klecka GM, Landi LP, Rodner KM (1985) Evaluation of the OECD Activated Sludge, Respiration Inhibition Test. Chemosphere 14, 1239-1251 (published)									
<b>1.2</b>	<b>Data protection</b>	No									
1.2.1	Data owner	-									
1.2.2	Criteria for data protection	No data protection claimed									
		2 GUIDELINES AND QUALITY ASSURANCE									
<b>2.1</b>	<b>Guideline study</b>	Yes. OECD guideline 209 'Activated sludge, respiration inhibition test' (1981)									
<b>2.2</b>	<b>GLP</b>	██████████									
<b>2.3</b>	<b>Deviations</b>	Yes. The synthetic sewage stock solution was prepared with increased level of K <sub>2</sub> HPO <sub>4</sub> (28 g instead of 2.8 g).									
		3 MATERIALS AND METHODS									
<b>3.1</b>	<b>Test material</b>	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.									
<b>3.2</b>	<b>Preparation of TS solution for poorly soluble or volatile test substances</b>	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.									
<b>3.3</b>	<b>Reference substance</b>	Yes. 3,5-Dichlorophenol as recommended in the guideline.									
3.3.1	Method of analysis for reference substance	No information									
<b>3.4</b>	<b>Testing procedure</b>										
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>K<sub>2</sub>HPO<sub>4</sub></td> <td>28 g</td> </tr> </tbody> </table>	Bacto-Peptide	16 g	Bacto-Beef extract	11 g	urea	3 g	K <sub>2</sub> HPO <sub>4</sub>	28 g	
Bacto-Peptide	16 g										
Bacto-Beef extract	11 g										
urea	3 g										
K <sub>2</sub> HPO <sub>4</sub>	28 g										



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

## Annex Point IIA7.4

MgSO <sub>4</sub> × 7 H <sub>2</sub> O	0.2 g
CaCl <sub>2</sub> × 2H <sub>2</sub> O	0.4 g
NaCl	0.7 g

Final pH of the stock solution was adjusted to pH 7.0 with H<sub>3</sub>PO<sub>4</sub>.

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-EC<sub>50</sub> >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**

	<b>reference substance</b>	
4.4.1	Concentrations	No information
4.4.2	Results	3 h EC <sub>50</sub> = 12.2 - 12.5 mg/L (3,5-dichlorophenol)
<b>5      APPLICANT'S SUMMARY AND CONCLUSION</b>		
<b>5.1</b>	<b>Materials and methods</b>	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 <sub>2</sub> HPO <sub>4</sub> (28 g instead of 2.8 mg).
<b>5.2</b>	<b>Results and discussion</b>	As result of the test a 3 h EC <sub>50</sub> >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 <sub>50</sub> range: 5-30 mg/L). The guideline study is well documented and the validity criteria are fulfilled.
5.2.1	EC <sub>20</sub>	-
5.2.2	EC <sub>50</sub>	3 h EC <sub>50</sub> >1000 mg/L
5.2.3	EC <sub>80</sub>	-
<b>5.3</b>	<b>Conclusion</b>	[REDACTED]
5.3.1	Reliability	[REDACTED]
5.3.2	Deficiencies	[REDACTED]

<b>Evaluation by Competent Authorities</b>	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
<b>EVALUATION BY RAPPORTEUR MEMBER STATE</b>	
<b>Date</b>	2008/07/03
<b>Materials and Methods</b>	[REDACTED]
<b>Results and discussion</b>	[REDACTED]
<b>Conclusion</b>	[REDACTED]
<b>Reliability</b>	[REDACTED]
<b>Acceptability</b>	[REDACTED]
<b>Remarks</b>	[REDACTED]

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

**Annex Point IIA7.4**

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

**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]

Reference:

[REDACTED]

<b>Section A7.4.2</b> <b>Annex Point II A 7.5</b>	<b>Bioconcentration, aquatic</b>
	[REDACTED]
<b>Undertaking of intended data submission</b> [ ]	[REDACTED]
<b>Evaluation by Competent Authorities</b>	
<i>Use separate "evaluation boxes" to provide transparency as to the comments and views submitted</i>	
<b>EVALUATION BY RAPPORTEUR MEMBER STATE</b>	
<b>Date</b>	2008/07/04
<b>Evaluation of applicant's justification</b>	[REDACTED]
<b>Conclusion</b>	[REDACTED]
<b>Remarks</b>	[REDACTED]
<b>COMMENTS FROM OTHER MEMBER STATE</b> <i>(specify)</i>	
<b>Date</b>	<i>Give date of comments submitted</i>
<b>Evaluation of applicant's justification</b>	<i>Discuss if deviating from view of rapporteur member state</i>
<b>Conclusion</b>	<i>Discuss if deviating from view of rapporteur member state</i>
<b>Remarks</b>	

**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***

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

5.2.1	LC <sub>0</sub>	[REDACTED]	
5.2.2	LC <sub>50</sub>	[REDACTED]	
5.2.3	LC <sub>100</sub>	[REDACTED]	
<b>5.3</b>	<b>Conclusion</b>	[REDACTED]	
5.3.1	Other Conclusions	[REDACTED]	
5.3.2	Reliability	[REDACTED]	
5.3.3	Deficiencies	[REDACTED]	

<b>Evaluation by Competent Authorities</b>	
<i>Use separate "evaluation boxes" to provide transparency as to the comments and views submitted</i>	
<b>EVALUATION BY RAPPORTEUR MEMBER STATE</b>	
<b>Date</b>	2014/02/12
<b>Materials and Methods</b>	[REDACTED]
<b>Results and discussion</b>	[REDACTED]
<b>Conclusion</b>	[REDACTED]
<b>Reliability</b>	[REDACTED]
<b>Acceptability</b>	[REDACTED]
<b>Remarks</b>	[REDACTED]
<b>COMMENTS FROM ...</b>	
<b>Date</b>	<i>Give date of comments submitted</i>
<b>Materials and Methods</b>	<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>
<b>Results and discussion</b>	<i>Discuss if deviating from view of rapporteur member state</i>
<b>Conclusion</b>	<i>Discuss if deviating from view of rapporteur member state</i>
<b>Reliability</b>	<i>Discuss if deviating from view of rapporteur member state</i>
<b>Acceptability</b>	<i>Discuss if deviating from view of rapporteur member state</i>
<b>Remarks</b>	

<b>Section A7.4.3.2</b>		<b>Effects on reproduction and growth rate of fish</b>	
Annex Point IIIA XIII 2.2			
<b>JUSTIFICATION FOR NON-SUBMISSION OF DATA</b>			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;"></div> <div style="background-color: black; width: 100%; height: 15px;"></div> <div style="background-color: black; width: 100%; height: 15px;"></div> <div style="background-color: black; width: 100%; height: 15px;"></div> <div style="background-color: black; width: 100%; height: 15px;"></div> <div style="background-color: black; width: 100%; height: 15px;"></div> <div style="background-color: black; width: 100%; height: 15px;"></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>		
<b>Evaluation by Competent Authorities</b>			
<i>Use separate "evaluation boxes" to provide transparency as to the comments and views submitted</i>			
<b>EVALUATION BY RAPPORTEUR MEMBER STATE</b>			
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

4.1.2	Number/ percentage of animals showing adverse effects	Not applicable	
4.1.3	Nature of adverse effects	Not applicable	
<b>4.2</b>	<b>Results test substance</b>		
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]	
<b>4.3</b>	<b>Results of controls</b>	[REDACTED]	x
<b>4.4</b>	<b>Test with reference substance</b>	Potassium dichromate	
4.4.1	Concentrations	No data	
4.4.2	Results	48 h EC <sub>50</sub> = 0.141 mg/L	
<b>5      APPLICANT'S SUMMARY AND CONCLUSION</b>			
<b>5.1</b>	<b>Materials and methods</b>	[REDACTED]	