Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA VII.7.3 *Scenedesmus Subspicatus*

			Official use only
		1 REFERENCE	
1.1	**Reference**	A. Ritter, 1989, Toxicity of Euparen WG 50 to *Scenedesmus Subspicatus* (OECD – Algae Growth Inhibition Test), RCC Umweltchemie AG, Itingen, Switzerland, RCC Project No. 235260 (unpublished),1989-05-25	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer Crop Science AG	
1.2.2	Companies with letter of access	Bayer Chemicals AG	
1.2.3	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/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OECD guideline No. 201	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Euparen WG 50, technical grade	
3.1.1	Lot/Batch number	Batch number: [redacted]	
3.1.2	Specification		X
3.1.3	Purity	Formulation with [redacted] active ingredient dichlofluanid.	X
3.1.4	Composition of Product	Investigation was performed with Euparen WG 50, technical grade containing [redacted] dichlofluanid.	X
3.1.5	Further relevant properties	-	
3.1.6	Method of analysis	After extraction with dichloromethane the combined extracts were evaporated to dryness. The residue was dissolved in toluene and analysed by GC.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Euparen WG 50 was suspended in the test medium at a concentration of 100 mg/100 ml (stock suspension). Thereafter series of sequential dilutions with the test medium were prepared to obtain the final test concentrations of the test substance.	
3.3	**Reference substance**	Yes, Potassium dichromate ($K_2Cr_2O_7$)	
3.3.1	Method of analysis for reference substance	No data	X
3.4	**Testing procedure**		

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA VII.7.3 *Scenedesmus Subspicatus*

3.4.1	Culture medium	The algae were cultivated in a nutrient solution prepared according to reference 2 (NEN 6506) of OECD guideline No. 201	
3.4.2	Test organisms	see table A7_4_1_3-1	
3.4.3	Test system	see table A7_4_1_3-2	
3.4.4	Test conditions	see table A7_4_1_3-3	X
3.4.5	Duration of the test	96 hours	
3.4.6	Test parameter	Influence on the growth	
3.4.7	Sampling	Samples to determine the number of algae/ml suspension were taken at 24, 48, 72 and 96 hours. pH of test medium was controlled at the beginning of the test and after 24, 48, 72 and 96 hours.	
3.4.8	Monitoring of TS concentration	Yes, Concentration of test substance in the test medium was determined for control, 0.016, 2 and 50 µg/ml. Samples of 5 ml were taken after 0 hours.	X
3.4.9	Statistics	The test results were evaluated by Logit – analysis, the Dunnett-Test was used for statistics.	

4 RESULTS

4.1	**Limit Test**	Not performed	
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	Nominal concentrations: 0.016, 0.080, 0.4, 2.0, 10.0 and 50.0 mg/l	
4.2.2	Actual concentrations of test substance	Measured concentrations of Euparen WG 50 (mg/l):	X

Nominal concentration (mg/l)	0 hours
Control	-/-
0.016	0.019 / 0.024
2.0	1.510 / 1.637
50.0	46.06 / 57.79

4.2.3	Growth curves	Growth curves (number of cells vs. time) are given in the report on page 25	

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA VII.7.3 *Scenedesmus Subspicatus*

4.2.4	Concentration / response curve	No growth inhibition curve represented.	
4.2.5	Cell concentration data	see table A7_4_1_3-4	
4.2.6	Effect data (cell multiplication inhibition)	EC_{50} values after 72 and 96 hours were 21.5 mg/l and 32.7 mg/l, respectively. Based on the growth inhibition curve, the NOEC and LOEC values for Euparen WG 50 after 96 hours were 2 mg/l and 10 mg/l, respectively.	X
4.2.7	Other observed effects	-	
4.3	**Results of controls**	Number of cells (x 10000) per ml (mean values): 0 hours 1 24 hours 4.02 48 hours 12.10 72 hours 101.55 96 hours 290.90	
4.4	**Test with reference substance**	Performed	
4.4.1	Concentrations	Control, 0.6, 1.0, 1.4, 1.8 and 2.2 mg/l	
4.4.2	Results	The EC_{50} value for potassium dichromate was 0.7 mg/l	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The influence of Euparen WG 50 on the growth of the green alga *Scenedesmus subspicatus* was investigated in a 96 hours static test according to OECD guideline No. 201. The test shows no significant deviations from the guideline.	
5.2	**Results and discussion**	The EC_{50} values after 72 and 96 hours were 21.5 mg/l and 32.7 mg/l, respectively. Based on the growth inhibition curve, the NOEC and LOEC values for Euparen WG 50 after 96 hours were 2 mg/l and 10 mg/l, respectively.	X
5.2.1	NOEC	2 mg/l after 96 hours	
5.2.2	EC_{50}	21.5 mg/l after 72 hours and 32.7 mg/l after 96 hours	
5.3	**Conclusion**	Validity criteria are summarised in table A7_4_1_3-5. The control cultures fulfil the validity criteria concerning the cell concentration. Dose – response relationship: a clear dose – response relationship cannot be derived from the cell concentration data.	
5.3.1	Reliability	2	
5.3.2	Deficiencies	Yes	

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA VII.7.3 *Scenedesmus Subspicatus*

The determination of test substance concentrations in the test medium was only performed with samples taken after 0 hours.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	10/08/06
Materials and Methods	Accept applicant's version noting the following minor deviations: **3.1.2** Specification of Euparen WG 50 has now been provided, and is included in the Confidential Annex of the dossier. **3.1.3** The purity of the test substance; Euparen WG 50, technical grade was only [redacted] dichlofluanid. **3.1.4** Percentage of ingredient only given for dichlofluanid. **3.3.1** No method of analysis given for reference substance. The information in the study is limited. **3.4.4** No data on aeration of test water. **3.4.8** There were only 3 measured concentrations from day 0, 2 measurements were made for each of the 3 concentrations 0.016, 2.0 and 50 mg/l. This deficiency is identified by the applicant in 5.3.2.
Results and discussion	Accept Applicant's version with the addition of the following: **4.2.2** The measured concentrations for the nominal of 2.0 mg/l were lower than the nominal at 1.510/1.637 mg/l (75.7 and 81.8 % respectively) and as the measurements were only made at day 0, the EC_{50}, NOEC and LOEC values, stated in 4.2.6, are all potentially underestimates for dichlofluanid. **4.2.6** The results refer to mg/l of Euparen WG 50 which only contains 50.7 % dichlofluanid, although the Applicant recalculated the endpoints to represent mg a.s./l in Doc II-A 4.2.1.3. **5.2** The results were adjusted by the Applicant to account for a.s. ([redacted] of formulation) and initial measured concentrations with EC_{50} values after 72 and 96 hours were 10.8 mg a.s./l and 16.4 mg a.s./l respectively. Based on the growth inhibition curve, the NOEC and LOEC values for dichlofluanid after 96 hours were 1 mg a.s./l and 5 mg a.s./l, respectively. However, the EC_{50} and NOEC values given by the Applicant are not based on growth rate but biomass and following TMII06, further re-calculations were requested from the Applicant on growth rate.

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA VII.7.3 *Scenedesmus Subspicatus*

These are reported below;

Endpoint	mg a.s/l
E_rC_{50} 24 h	n.d
E_rC_{50} 48 h	261.2
E_rC_{50} 72 h	15.0
E_rC_{50} 96 h	22.3
NOEC 96 h	1.0

n.d - not determined due to mathematical reasons

The UK CA has reconsidered the data from the original report which show that there was no significant inhibitory effect on growth rate after 24 hours (presented in Figure 1) for all concentrations tested. It can be considered that any acute effect would have been seen during the 24-hour period, as this is where the concentrations of the a.s. would have been significant. The UK CA further considers that whilst the test concentrations would have continued to decrease after 24 hours, the fact that inhibitory effects on growth rate were only visible in the highest nominal concentration tested (after 48 hours) gives some degree of confidence in the NOE_rC of 1 mg a.s./l being sufficiently protective without the need for time weighted average calculations. This argument was accepted at TMI06 and is further justified because the data available clearly show that dichlofluanid is far more toxic to both aquatic invertebrates and fish (by an order of magnitude), and a repeated algal study for this product type is not considered necessary.

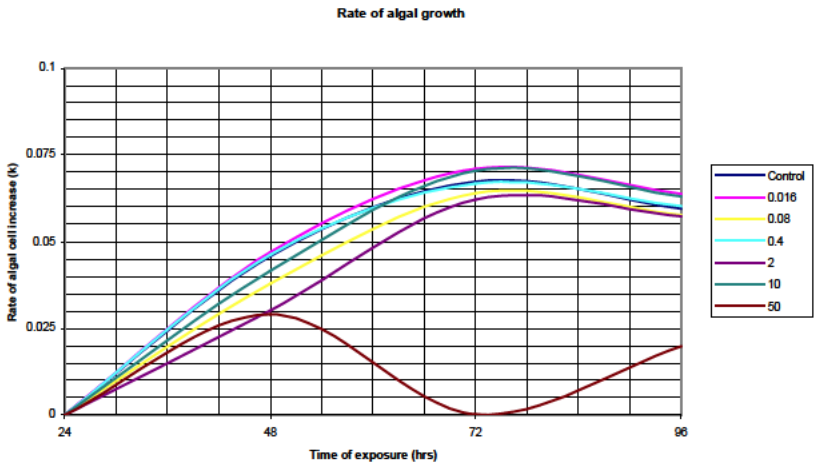

Figure 1: Algal growth curves following exposure to dichlofluanid (initial nominal concentrations given in mg/l)

Dichlofluanid showed limited toxicity to algae. In the risk assessment for algae the E_rC_{50} values of 15.0 mg/l and a NOEC of 1.0 mg/l are used based on nominal concentrations.

Conclusion Accept Applicant's version noting the recalculated endpoints

Reliability 2

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA VII.7.3 *Scenedesmus Subspicatus*

Acceptability	Acceptable The guideline does not specify measured concentrations, therefore the deficiencies are not considered significant enough to justify repeating the study since there are data to show that the fish and aquatic invertebrate species are more sensitive.
Remarks	All endpoints and data presented in the summary and tables have been checked against the original summary and are correct.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_4_1_3-1: Test organisms

Criteria	Details
Species	Green alga *Scenedesmus subspicatus chodat*
Strain	-
Source	Test organism was supplied by the Umweltbundesamt Berlin, Germany and were grown in the laboratories of RCC Umweltchemie AG
Laboratory culture	Yes
Method of cultivation	Standardised conditions
Pre-treatment	Cells for the test were taken from a pre-culture which was set up to 72 hours prior to the test with the same conditions as the test
Initial cell concentration	Test started with a biomass of 10´000 cells per ml nutrient solution

Table A7_4_1_3-2: Test system

Criteria	Details
Volume of culture flasks	50 ml Erlenmeyer flasks
Culturing apparatus	Shaking water bath at 22-23.5 °C with continuous illumination at 8000 Lux
Light quality	4 fluorescent tubes (Philips TL 33, 20 W, 60 cm)
Procedure for suspending algae	Shaking (120 strokes/min.)
Number of vessels/ concentration	3
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_3-3: Test conditions

Criteria	Details
Test temperature	22-23.5 °C
pH	At the beginning of the test the pH of the test medium was adjusted to 7.6. The mean values of the pH during the test were in the range of 7.1-8.0.
Aeration of dilution water	No data
Light intensity	8000 Lux
Photoperiod	Continuous illumination

Table A7_4_1_3-4: Cell concentration data

Test Substance Concentration (nominal)[1] [mg/l]	**Cell concentrations (mean values) [cells/ml][2]**							
	measured				**Percent of control**			
	24 h	**48 h**	**72 h**	**96 h**	**24 h**	**48 h**	**72 h**	**96 h**
control	4.02	12.10	101.55	290.90	100	100	100	100
0.016	2.93	9.03	88.70	287.40	73	75	87	99
0.080	4.37	10.87	94.53	276.50	109	90	93	95
0.4	3.47	10.53	85.37	265.40	86	87	84	91
2.0	4.70	9.67	92.50	289.63	117	80	91	100
10.0	2.53	6.77	73.63	234.20	63	56	73	81
50.0	2.87	5.77	2.93	12.03	71	48	3	4
Temperature [°C]	*	*	*	*				
pH** (mean value)	7.8	7.9	8.0	7.1				

[1] Test substance concentrations are nominal concentrations

[2] number of cells per ml (divided by 10`000 and corrected for N0 = 10`000)

* Test temperature was 22-23.5 °C

** pH at the beginning was 7.7

Table A7_4_1_3-5: Validity criteria for algal growth inhibition test according to OECD Guideline 201

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

Criteria for poorly soluble test substances	-	-