Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA VII.7.3 **DIMETHYLAMINOSULFANILID (DMSA)**

Scenedesmus subspicatus

			Official use only
		1 REFERENCE	
1.1	**Reference**	N. Casper, 1997, Dimethylaminosulfanilid (DMSA) Alga, Growth Inhibition Test, Bayer AG, Institute for Environmental Analysis, Leverkusen, Germany, Report No. 689 A/97 A1 (unpublished), 1997-12-08	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer 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, Council Directive 92/69/EEC, C.3. This method is in most parts identical with OECD guideline No. 201	
2.2	**GLP**	Yes	
2.3	**Deviations**	No, the study is comparable to OECD guideline No. 201	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Dimethylaminosulfanilid (DMSA)	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification		X
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	-	
3.1.5	Further relevant properties	water solubility = 2 g/l at 20 °C	
3.1.6	Method of analysis	No data	X
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	The test substance was added directly to the test water without the use of solvents and distributed by ultrasonic bath and magnetic stirrer.	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	-	

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3.4	**Testing procedure**		
3.4.1	Culture medium	No data	X
3.4.2	Test organisms	see table A7_4_1_3-1	
3.4.3	Test system	see table A7_4_1_3-2	X
3.4.4	Test conditions	see table A7_4_1_3-3	
3.4.5	Duration of the test	72 hours	
3.4.6	Test parameter	Inhibition of growth	
3.4.7	Sampling	The cell concentration in each flask is determined at 24, 48 and 72 hours after the start of the test. The pH is measured at the beginning of the test and at 72 hours.	X
3.4.8	Monitoring of TS concentration	Yes, at the beginning and at the end of the test	
3.4.9	Statistics	Results (EC_0) were determined directly from the study	
		4 RESULTS	
4.1	**Limit Test**	Performed	
4.1.1	Concentration	100 mg/l	X
4.1.2	Number/ percentage of animals showing adverse effects	-	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	Nominal concentration: 100 mg/l (limit test)	X
4.2.2	Actual concentrations of test substance	Measured concentrations: 96.6 mg/l at 0 hours, 98.7 mg/l at 72 hours, Average: 97.7 mg/l	X
4.2.3	Growth curves	Growth curves are given in the report on page 17	
4.2.4	Concentration / response curve	No concentration/response curve given	
4.2.5	Cell concentration data	see table A7_4_1_3-4	
4.2.6	Effect data (cell multiplication inhibition)	The test substance dimethylaminosulfanilid has no toxic effects against algae at an analytical concentration of 97.7 mg/l. ($EC_0 \geq 97.7$ mg/l)	
4.2.7	Other observed	-	

	effects	
4.3	**Results of controls**	Number of cells (x 10000) per ml:
		0 hours 1
		24 hours 7.67
		48 hours 32.20
		72 hours 50.10
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	-
4.4.2	Results	-

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	To assess the acute toxic effects of dimethylaminosulfanilid (DMSA) on the growth and on the growth rate of the algal species *Scenedesmus subspicatus*, a 72-hour test was performed. The study was conducted in accordance with the Council Directive 92/69/EEC, C.3, which is in most parts identical with the OECD guideline No. 201. The test shows no significant deviations from the guideline.
5.2	**Results and discussion**	The test substance dimethylaminosulfanilid has no toxic effects against algae at an analytical concentration of 97.7 mg/l ($EC_0 \geq 97.7$ mg/l). The test substance was sufficiently stable under the test conditions. The analytical data show that the test concentration was over 80% of the theoretical value of 100 mg/l throughout the duration of the test.
5.2.1	NOE_rC	-
5.2.2	E_rC_{50}	-
5.2.3	E_bC_{50}	-
5.3	**Conclusion**	Validity criteria are summarized in table A7_4_1_3-5. The test fulfils the validity criteria of the OECD guideline No. 201. Dose response-relationship: the resulting cell concentrations measured for the test substance level at the different time points are higher than the cell concentrations determined for the control.
5.3.1	Reliability	2
5.3.2	Deficiencies	Yes, Some reporting deficiencies: no information about the composition of the culture medium; Information incomplete about the test system; no method of analysis mentioned used for the determination of the test

substance concentration in the test vessel;

no method mentioned used for the measurement of the cell concentration

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Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	28/01/05
Materials and Methods	Accept applicant's version noting the following deviations: **3.1.2** There is no specification given for the test substance. **3.1.6** There is no method of analysis given for the test substance, although as **3.4.8** states; the concentrations of the test substances have been monitored. This is identified as a deficiency by the applicant in 5.3.2. **3.4.1** There is no description of the culture medium given. This is highlighted as a deficiency in the conclusion by the applicant. **3.4.3** As identified by the applicant in the conclusion the information about the test system is incomplete. There is no information on the number of replicates used for the test concentration and the control, from the study it appears that only one replicate was used for the concentration and one for the control. There is no information about the photoperiod, although the study indicates continuous illumination. **3.4.7** There is no method referred to for the determination of cell concentrations. This is identified as a deficiency by the applicant in the 5.3.2.
Results and discussion	Accept applicant's version with the following comment: **4.1.1** and **4.2.1** There is only one test concentration, the test is a limit test.
Conclusion	Accept applicant's version
Reliability	Reliability = 2
Acceptability	Acceptable Deficiencies are not considered significant enough to justify repeating the study.
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*

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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	Origin of the test organism: Institute of Plant Physiology, University of Goettingen, Germany
Laboratory culture	Yes
Method of cultivation	Cultivation of stock cultures, pre - cultures and test cultures took place in a light chamber at 23 $\pm$ 2 °C and with a quantum flux which equals 120 µE/m^2 × s
Pretreatment	-
Initial cell concentration	Test started with a biomass of 10´000 cells

Table A7_4_1_3-2: Test system

Criteria	**Details**
Volume of culture flasks	-
Culturing apparatus	Light chamber at 23 $\pm$ 2 °C and with a quantum flux which equals 120 µE/m^2 × s
Light quality	-
Procedure for suspending algae	shaking
Number of vessels/ concentration	-
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_3-3: Test conditions

Criteria	Details		
Test temperature	23 ± 2 °C		
pH	Concentration of test substance (mg/l)	pH value	
		0 hours	72 hours
	Control	8.4	10.4
	100	8.0	9.9
Aeration of dilution water	No data		
Light intensity	quantum flux which equals 120 $\mu E/m^2 \times s$		
Photoperiod	-		

Table A7_4_1_3-4: Cell concentration data

Test-Substance Concentration (nominal) [mg/l]	Cell concentration [cells/ml]							
	measured				Percent of control			
	0h	24h	48h	72h	0 h	24 h	48 h	72 h
Control	10000	76700	322000	501000	100	100	100	100
100	10000	83300	349000	538000	100	109	108	107
Temperature [°C]	*	*	*	*				
pH	**	-	-	**				

* Test temperature was 23 ± 2 °C

** see table 7_4_1_3-3 Test conditions

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	**X**	

Criteria for poorly soluble test substances	-	-