Section A7.4.1.2

Annex Point IIA VII.7.2

Acute toxicity to invertebrates of

DIMETHYLAMINOSULFANILID (DMSA)
Daphnia magna

			Official use only
	1 **REFERENCE**		
1.1	**Reference**	N. Caspers, 1997, Dimethylaminosulfanilid (DMSA) Acute Daphnia Toxicity, Bayer AG, Institute for Environmental Analysis, Leverkusen, Germany, Report No. 689 A/97 D (unpublished), 1997-11-27	
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,	
		Study was performed in accordance with Council Directive 92/69/EEC, Part C.2. This test method is in most parts identical with OECD guideline No. 202	
2.2	**GLP**	Yes	
2.3	**Deviations**	No,	
		the study is comparable to OECD guideline No. 202	
	3 **MATERIALS AND METHODS**		
3.1	**Test material**	Dimethylaminosulfanilid (DMSA)	
3.1.1	Lot/Batch number	██████████████	
3.1.2	Specification		
3.1.3	Purity	████	
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	-	
3.4	**Testing procedure**		

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3.4.1	Dilution water	see table A7_4_1_2-1	X
3.4.2	Test organisms	see table A7_4_1_2-2	X
3.4.3	Test system	see table A7_4_1_2-3	X
3.4.4	Test conditions	see table A7_4_1_2-4	X
3.4.5	Duration of the test	48 hours	
3.4.6	Test parameter	Immobilisation	
3.4.7	Sampling	Immobilisation of Daphnia is recorded at the start, after 24 hours and at the end of the study.	X
		Water temperature, pH and oxygen values are measured at the end of the study.	
		The concentrations of the C-containing components of the test medium were confirmed by TOC determination at the start and end of the study.	
3.4.8	Monitoring of TS concentration	Yes, at the start and end of the test	
3.4.9	Statistics	The EC_0 was determined directly from the study	

4   RESULTS

4.1	**Limit Test**	Performed	
4.1.1	Concentration	100 mg/l	
4.1.2	Number/ percentage of animals showing adverse effects	No immobilisation of daphnids occurred in the test level.	
4.1.3	Nature of adverse effects	-	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	Nominal concentration: 100 mg/l (limit test)	
4.2.2	Actual concentrations of test substance	Measured concentrations: 94.5 mg/l at 0 hours, 96.6 mg/l at 48 hours, Average: 95.6 mg/l	
4.2.3	Effect data (Immobilisation)	see table A7_4_1_2-5 and table A7_4_1_2-6	
4.2.4	Concentration / response curve	No immobilisation occurred during the test. Therefore no concentration / response curve is given in the report.	
4.2.5	Other effects	-	

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4.3	**Results of controls**	No immobilisation occurred in the control
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 (immobilisation) of dimethylamino-sulfanilid (DMSA) on *Daphnia magna*, a 48-hour limit test under static conditions was performed.
		The study was conducted in accordance to the Council Directive 92/69/EEC, C.2, which is in most parts identical with the OECD guideline No. 202.
		Comparison with OECD guideline No. 202 shows no relevant deviations.
5.2	**Results and discussion**	The EC_0 of the test substance dimethylaminosulfanilid (DMSA) after 48 hours for the species *Daphnia magna* is ≥ 95.6 mg/l.
		No immobilisation occurred in the control and the 100 mg/l test level.
		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	EC_0	≥ 95.6 mg/l after 48 hours
5.2.2	EC_{50}	-
5.2.3	EC_{100}	-
5.3	**Conclusion**	The validity criteria are summarised in table A7_4_1_2-7.
		The test fulfils the validity criteria of the OECD guideline No. 202.
5.3.1	Reliability	2
5.3.2	Deficiencies	Yes
		Information incomplete about dilution water, test organism, test system and test conditions.
		No method of analysis mentioned used for determination of the test substance concentration in the test vessel.

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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	13/12/04
Materials and Methods	Accept applicant's version noting the following minor deviations:
	3.1.6 No method of analysis mentioned for the determination of the test substance concentration in the test vessel, as highlighted in 5.3.2.
	3.4.1-**3.4.4** There is incomplete information about dilution water, test organism, test system and test conditions, as highlighted in 5.3.2. Details about the dilution water are limited to the source and hardness. Details about the test organisms do not include any description of food /feeding or pre-treatment. Details about the test system do not include any description of the volume of the vessels or the Volume/animal. There is no description of the photoperiod in the test conditions.
	3.4.3 Ten daphnids in one vessel were used for the control and 10 in another vessel were used for the test, as opposed to 20 divided into 4 groups for each concentration and the control.
	3.4.7 Water temperature, pH and oxygen values only measured at the end of the study not every 24 h. Sample storage times before analysis was not given.
Results and discussion	Accept applicant's version
Conclusion	Accept applicant's version
Reliability	Reliability = 2
Acceptability	Acceptable
	The deficiencies are considered to be minor.
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_2-1:   **Dilution water**

Criteria	Details
Source	M4-medium according to BGA (1992)
Alkalinity (CaCO$_3$)	-
Hardness (CaCO$_3$)	274.9 mg/l CaCO$_3$ = 15.4 d°H
pH	-
Ca / Mg ratio	-
Na / K ratio	-
Oxygen content	-
Conductance	-
Holding water different from dilution water	No

Table A7_4_1_2-2:   **Test organisms**

Criteria	Details
Strain	Daphnia magna STRAUS, parthenogenetic females
Source	Origin of daphnids: Bundesgesundheitsamt (BGA) Berlin, where they were cloned. Test animals were bred in the laboratory at Bayer AG, Leverkusen
Age (at start of the study)	0 – 24 hours
Breeding method	Keeping of Daphnia: M4-medium according to BGA (1992)
Kind of food	-
Amount of food	-
Feeding frequency	-
Pretreatment	-
Feeding of animals during test	No data

Table A7_4_1_2-3:   **Test system**

Criteria	Details
Renewal of test solution	Static test conditions
Volume of test vessels	-
Volume/animal	-
Number of animals/vessel	10
Number of vessels/ concentration	two parallels with 10 Daphnia each
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_2-4:   **Test conditions**

Criteria	Details
Test temperature	20.3 °C (control) and 20.1 °C (test level) after 48 hours
Dissolved oxygen	9.3 mg/l (control) and 9.5 mg/l (test level) after 48 hours
pH	7.7 (control) and 7.9 (test level) after 48 hours
Adjustment of pH	No
Aeration of dilution water	No data
Quality/Intensity of irradiation	-
Photoperiod	-

Table A7_4_1_2-5:   **Immobilisation data**

| Test-Substance Concentration (nominal) [mg/l] | Immobile *Daphnia* | | | | Oxygen [mg/l] | pH | Temperature [°C] |
| | Number | | Percentage | | | | |
	24 h	48 h	24 h	48 h	48 h	48 h	48 h
Control	0	0	0	0	9.3	7.7	20.3
100	0	0	0	0	9.5	7.9	20.1

Table A7_4_1_2-6:   **Effect data**

	$EC_{50}{}^{1}$	95 % c l.	$EC_0{}^{1}$	$EC_{100}{}^{1}$
24 h [mg/l]	-	-	-	-
48 h [mg/l]	-	-	≥ 95.6	-

[1] Effect data are based on measured concentrations

Table A7_4_1_2-7:   **Validity criteria for acute daphnia immobilisation test according to OECD Guideline 202**

	fulfilled	Not fulfilled
Immobilisation of control animals <10%	**X**	
Control animals not staying at the surface	**X**	
Concentration of dissolved oxygen in all test vessels >3 mg/l	**X**	
Concentration of test substance $\geq$ 80% of initial concentration during test	**X**	

Criteria for poorly soluble test substances	-	-