

Section A7.4.3.1 Prolonged toxicity to an appropriate species of fish**Annex Point IIIA XIII.2.1 DIMETHYLAMINOSULFANILID (DMSA)**

		1 REFERENCE	
1.1 Reference		[REDACTED], 1990, Toxicity of DMSA for Rainbow Trout (<i>Oncorhynchus Mykiss</i>) with prolonged exposure (21 days), [REDACTED] Report No. [REDACTED] (unpublished), 1990-07-18	
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. 204	
2.2 GLP		Yes	
2.3 Deviations		No	
		3 MATERIALS AND METHODS	
3.1 Test material		Dimethylaminosulfanilid (DMSA)	
3.1.1 Lot/Batch number		Lot number: [REDACTED]	
3.1.2 Specification			
3.1.3 Purity		[REDACTED]	
3.1.4 Composition of Product		-	
3.1.5 Further relevant properties		Water solubility: 1.3 g/l (20 °C, distilled water)	
3.1.6 Method of analysis		HPLC	
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 as evenly as possible by stirring.	
3.3 Reference substance		No	
3.3.1 Method of analysis for reference substance		-	
3.4 Testing procedure			
3.4.1 Dilution water		see table A7_4_3_1-1	
3.4.2 Test organisms		see table A7_4_3_1-2	

Official
use only

Section A7.4.3.1 Prolonged toxicity to an appropriate species of fish**Annex Point IIIA XIII.2.1 DIMETHYLAMINOSULFANILID (DMSA)**

3.4.3	Test system	see table A7_4_3_1-3
3.4.4	Test conditions	see table A7_4_3_1-4
3.4.5	Duration of the test	21 days
3.4.6	Test parameter	Mortality and observable symptoms
3.4.7	Sampling	<p>The fish were examined for symptoms of intoxication and mortality every working day but at least three times per week (each test level and control).</p> <p>The oxygen concentration and the pH were measured every working day in each aquarium.</p> <p>Body weight and length of the fish were measured for a representative random sample of each batch of fish used before the start of the experiment and for all surviving fish at the end of the experiment.</p>
3.4.8	Monitoring of TS concentration	<p>Yes,</p> <p>the test substance concentrations were analysed immediately before the start of the experiment, after 7 days shortly before changing the water, and after 21 days in the concentrations still containing surviving fish. Concentrations below the NOEC were not analysed.</p>
3.4.9	Statistics	<p>The NOEC was referenced to the most sensitive parameter (observed toxic symptoms or growth).</p> <p>Body weights and length of the surviving fish of the various test concentrations at the end of the experiment were compared with those of the controls using t-tests.</p>

4 RESULTS

4.1	Limit Test	Not performed
4.1.1	Concentration	-
4.1.2	Number/ percentage of animals showing adverse effects	-
4.1.3	Nature of adverse effects	-
4.2	Results test substance	
4.2.1	Initial concentrations of test substance	Nominal concentrations: 10 and 100.0 mg test substance per litre
4.2.2	Actual concentration of test substance	Actual concentrations of test substance see table A7_4_3_1-7
4.2.3	Effect data	see table A7_4_3_1-5 and table A7_4_3_1-6

X

Section A7.4.3.1 Prolonged toxicity to an appropriate species of fish**Annex Point IIIA XIII.2.1 DIMETHYLAMINOSULFANILID (DMSA)**

	(Mortality)		
4.2.4	Concentration / response curve	No graph available, since only one fish was found to be dead in the 100 mg/l test level at the end of the study period.	
4.2.5	Other effects	Observable symptoms were noted among the fish in the 100 mg/l test level. The body weights and length of the surviving fish in the 100 mg/l test concentration group at the end of the exposure time were statistically significantly different from those of the controls ($p \leq 0.05$).	X
4.3	Results of controls		
4.3.1	Number/ percentage of animals showing adverse effects	No deaths or behavioural changes occurred in the controls.	
4.3.2	Nature of adverse effects	-	
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	The toxicity of dimethylaminosulfanilid (DMSA, hydrolysis product of dichlofluanid) for rainbow trout (<i>Oncorhynchus mykiss</i>) with prolonged exposure was investigated in a 21-day semi - static experiment in accordance with the OECD guideline No. 204. The test shows no significant deviations from the guideline.	
5.2	Results and discussion	A 21 - day LC ₅₀ value was calculated to be > 100 mg test substance per litre. The lowest lethal concentration (LLC) and the LOEC were 100 mg/l, the NOEC was 10 mg/l. The results are based on the nominal concentrations of DMSA. No deaths or behavioural changes occurred in the controls. The body weights and length of the surviving fish in the 100 mg/l test concentration group at the end of the exposure time were statistically significantly different from those of the controls ($p \leq 0.05$). The test substance was sufficiently stable under the test conditions. The analytical data show that the test concentrations were over 80% of the theoretical value throughout the duration of the test.	
5.2.1	21d-LC ₅₀	> 100 mg/l	
5.2.2	NOEC	10 mg/l	
5.2.3	LOEC	100 mg/l	
5.3	Conclusion	The validity criteria are summarised in table A7_4_3_1-8.	

Section A7.4.3.1 Prolonged toxicity to an appropriate species of fish**Annex Point IIIA XIII.2.1 DIMETHYLAMINOSULFANILID (DMSA)**

The test fulfils the validity criteria of the OECD guideline No. 204.

Dose – response relationship: only one fish was found to be dead in the 100 mg/l test level at the end of the study period.

According to the results of this experiment, DMSA is far less toxic than dichlofluanid, from which it is formed by hydrolysis.

5.3.1	Other Conclusions	-
5.3.2	Reliability	1
5.3.3	Deficiencies	No

Section A7.4.3.1 Prolonged toxicity to an appropriate species of fish**Annex Point IIIA XIII.2.1 DIMETHYLAMINOSULFANILID (DMSA)**

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
Results and discussion	Accept applicant's version noting the following additions: 4.2.2 The analytical determination of concentration appears to show an increase in DMSA from the concentration on day 0. The determinations were carried out in duplicate, with the duplicate values being relatively close. 4.2.5 The applicant refers to sub-lethal effects in the 100 mg/l concentration, with reference to the study the effects observed are as follows: From day 2 - 21; Fish staying mainly at the bottom of the tank, sideways or inverted position, tumbling swimming, On day 8 fish staying mainly near the water surface was observed and on day 9 slight behavioural changes relative to the control were observed, From day 9 - 21 reduced food intake, Body weights and lengths of fish in this concentration were significantly different from the control.
Conclusion	Accept applicant's version
Reliability	Reliability = 1
Acceptability	Acceptable
Remarks	All endpoints and data presented in the summary and tables have been checked against the original summary and are correct.
	COMMENTS FROM ...
Date	<i>Give date of comments submitted</i>
Materials and Methods	<i>Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state</i>
Results and discussion	<i>Discuss if deviating from view of rapporteur member state</i>
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>
Reliability	<i>Discuss if deviating from view of rapporteur member state</i>
Acceptability	<i>Discuss if deviating from view of rapporteur member state</i>
Remarks	

Table A7_4_3_1-1: Dilution water

Criteria	Details
Source	Reconstituted oxygen – saturated water with the following ion concentrations was used (according to ISO): Ca ²⁺ = 0.384 mmol/l; Mg ²⁺ = 0.096 mmol/l; Na ⁺ = 0.148 mmol/l; K ⁺ = 0.015 mmol/l; Cl ⁻ = 0.783 mmol/l; HCO ₃ ⁻ = 0.148 mmol/l; SO ₄ ²⁻ = 0.096 mmol/l
Alkalinity	-
Hardness	40- 60 mg of CaCO ₃ /l
pH	7.4 (Control at day 0 of the test)
Oxygen content	11.5 mg/l (Control at day 0 of the test)
Conductance	-
Holding water different from dilution water	No

Table A7_4_3_1-2: Test organisms

Criteria	Details
Species/strain	Rainbow trout (<i>Oncorhynchus mykiss</i>)
Source	Test fish were obtained from the Linn trout farm, Lennestadt, Germany.
Wild caught	No
Age/size	The mean body weight of the fish at the start of the test was 1.26 ± 0.28 g and the mean body length was 4.8 ± 0.29 cm.
Kind of food	Commercial trout feed (Brutfutter FB50, Kronen-Fischkraftfutter)
Amount of food	The amount of feed corresponded to 2% dry mass of the starting body weight.
Feeding frequency	-
Pretreatment	The fish was acclimatised in the test water at the test temperature for at least 14 days.
Feeding of animals during test	Yes

Table A7_4_3_1-3: Test system

Criteria	Details
Test type	Semi - static
Renewal of test solution	Every 7 days, the fish were transferred into a clean aquarium with the relevant concentration.
Volume of test vessels	40 l
Volume/animal	4 l
Number of animals/vessel	10
Number of vessels/ concentration	1
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_3_1-4: Test conditions

Criteria	Details
Test temperature	15 ± 2 °C
Dissolved oxygen	9.2 – 12.6 mg/l
pH	6.9 – 7.5
Adjustment of pH	No
Aeration of dilution water	Yes (pretreatment)
Intensity of irradiation	-
Photoperiod	16 hours light/8 hours dark

Table A7_4_3_1-5: Mortality data

Day no.	Mortality (number of fish)		
	Test Substance: Nominal concentration [mg/l]		
	Control	10.0	100.0
1	0	0	0
2	0	0	0
5	0	0	0
6	0	0	0
7	0	0	0
8	0	0	0
9	0	0	0
12	0	0	0
13	0	0	0
14	0	0	0
16	0	0	0
19	0	0	0
20	0	0	0
21	0	0	1
Temperature [°C]	15 ± 2		
pH	6.9 – 7.5		
Oxygen [mg/l]	9.2 – 12.6		

Table A7_4_3_1-6: Effect data

	21 d [mg/l] ¹	95 % c.l.
LC ₅₀	> 100	-
NOEC	10	-
LOEC	100	-

¹ Effect data are based on nominal concentrations

Table A7_4_3_1-7: Actual concentrations of dimethylaminosulfanilid (DMSA)

Sampling time	Theoretical concentration of DMSA (Präp) (mg/l)	Actual concentration of DMSA (mg/l)		
		1. Detection	2. Detection	Average
Day 0	9.8 (10)	8.3	8.1	8.2
Day 7		10.5	10.3	10.4
Day 21		9.3	9.5	9.4
Day 0	98 (100)	82	82	82
Day 7		105	103	104
Day 21		105	106	106

Table A7_4_3_1-8: Validity criteria for prolonged fish test according to OECD Guideline 204

	fulfilled	Not fulfilled
Mortality of control animals <10%	X	
Concentration of dissolved oxygen in all test vessels > 60% saturation	X	
Concentration of test substance ≥80% of initial concentration during test	X	
Criteria for poorly soluble test substances	-	-