Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA, XIII.3.4 **DIMETHYLAMINOSULFANILID (DMSA)**

Chironomus riparius

Official use only

	1 REFERENCE	
1.1	**Reference**	F. Heimbach, 1999, Influence of Dimethylaminosulfanilid (DMSA) on Development and Emergence of Larvae of *Chironomus riparius* in a Water-Sediment System, BAYER AG, Institute for environmental biology, Report No. HBF/Ch 31 (unpublished), 1999-05-27
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" No guidelines available, however this study was done according to a proposal for a BBA-Guideline: "Effects of plant protection products on the development of sediment-dwelling larvae of *Chironomus riparius* in a water-sediment system" (1995)
2.2	**GLP**	Yes
2.3	**Deviations**	No
	3 MATERIALS AND METHODS	
3.1	**Test material**	Dimethylaminosulfanilid (DMSA)
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	
3.1.3	Purity	[redacted]
3.1.4	Composition of Product	-
3.1.5	Further relevant properties	-
3.1.6	Method of analysis	For chemical analysis of the active ingredient additional parallel replicates were prepared for analytical purposes only (control: one replicate; 0.10, 1.0 and 10 mg pure metabolite/l: two replicates). Three times during the study (1 hour, 7 and 28 days after application) one test container of the nominal concentrations of 0.10, 1.0 and 10 mg pure metabolite/l each was removed from the study (1 hour and 7 days after application the parallel replicates were taken). The overlying water of these test containers was carefully decanted. The sediment of each beaker was filtered by vacuum. The filtrate (= pore water) and the overlying water were analysed by HPLC. In addition the overlying water and pore water of the control were also analysed.

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3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	539 mg test substance were given to 2000 ml test water to obtain stock solution I and 100 ml from this stock solution were made up to 1000 ml with test water to make up stock solution II. Each solution was stirred on a magnetic stirrer for 15 minutes. In order to reach the nominal test concentrations appropriate volumes of stock solution I and II respectively were applied into the overlying water column of the beakers. Aliquots of the stock solutions (1.0 to 1000 ml) were applied just below the water surface with a pipette. Gentle mixing of the water ensured homogeneous distribution without disturbance of the sediment.	
3.3	**Reference substance**	Not performed	
3.3.1	Method of analysis for reference substance	-	
3.4	**Testing procedure**		
3.4.1	Dilution water, Test sediment	Details on Dilution water see table A7_4_1_2-1. The test sediment used was artificial sediment which was prepared 8 days before the start of the test. It consists of 69% fine quartz sand, 10% dried, finely ground peat, 20% kaolin and around 1% calcium carbonate. Details on test sediment see table A7_4_1_2-1a	
3.4.2	Test organisms	see table A7_4_1_2-2	
3.4.3	Test system	see table A7_4_1_2-3	
3.4.4	Test conditions	see table A7_4_1_2-4	X
3.4.5	Duration of the test	28 days	
3.4.6	Test parameter	The sex, time of emergence and number of emerged midges	
3.4.7	Sampling	The test vessels were observed at least three times per week to make a visual assessment of any behavioural differences compared to the control. The sex, time and number of emerged or not fully emerged adults were recorded daily during the period of emergence. Once per week samples of the water column of each container were taken and the pH and the dissolved oxygen content were measured. Temperature was measured in some beakers only.	
3.4.8	Monitoring of TS concentration	Yes, the concentration of dimethylaminosulfanilid (DMSA) was analysed in the overlying water and the pore water of the sediment in the test containers with the nominal test substance concentrations 0.10, 1.0 and 10 mg pure metabolite/l three times during the study (1 hour, 7 and 28 days after application). In addition the overlying water and the pore water of the control were also analysed on day 0.	
3.4.9	Statistics	Statistical analysis was obtained by employing a computerised program. χ^2-test was performed to establish different sensitivities of sexes and probit analysis was performed to calculate the EC_{15} and EC_{50} for	

		numbers of emerged midges.	
		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: 0.010, 0.032, 0.10, 0.32, 1.0, 3.2, 10, 32 and 100 mg pure metabolite/l	
4.2.2	Actual concentrations of test substance	Actual concentrations see table A7_4_1_2-7	X
4.2.3	Effect data	Effect data see table A7_4_1_2-5. In the highest concentration (100 mg pure metabolite/l) no adult midges emerged. The influence on the development after 28 days (EC_5, EC_{10}, EC_{15} and EC_{50}) is shown in table A7_4_1_2-6.	
4.2.4	Concentration / response curve	Dose-effect-curves on number of emerged midges (sum of male and female midges) and on the development rate of the sum of male and female midges are given in the report on page 25.	
4.2.5	Other effects	Abnormal behaviour of larvae, pupae or midges was not observed throughout the study.	
4.3	**Results of controls**	81% of the inserted larvae maturated to adults in the control	X
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 influence of dimethylaminosulfanilid (DMSA) on development and emergence of larvae of *Chironomus riparius* in a water-sediment system a study was performed according to the BBA-Guideline "Effects of plant protection products on the development of sediment-dwelling larvae of *Chironomus riparius* in a water-sediment system" (1995). In a 28-day static test system larvae of *Chironomus*	

		riparius were exposed to different concentrations of the test substance. The test shows no significant deviations from the BBA-Guideline.
5.2	**Results and discussion**	During the study, the concentrations of dimethylaminosulfanilid (DMSA) in the test water were analysed on day 0, 7 and 28 at the nominal concentrations of 0.10, 1.0 and 10 mg pure metabolite/l. The results of the test concentrations 0.10, 1.0 and 10 mg pure metabolite/l were 90.0 to 97.0% of nominal (on average 93.7%) one hour after application. These findings prove that the nominal concentrations of this study were prepared correctly and nominal concentrations can be used to calculate EC-values. The concentrations of the pure metabolite in the overlying water declined only insignificantly during the study. The results of the test concentrations 0.10, 1.0 and 10 mg pure metabolite/l were 79.0 to 84.0% of nominal (on average 81.0%) on day 28. The summary of numbers of emerged midges over 28 days is given in Table A7_4_1_2-5. Accordingly, 81% of the inserted larvae maturated to adults in the control, fulfilling the guideline requirements, and 72 to 86% emerged in the nominal concentrations of 0.01 to 32 mg pure metabolite/l. In the highest concentration (100 mg pure metabolite/l) no adult midges emerged. The χ^2-test established no difference of sex in emerged midges at any test concentration ($p = 0.05$). Because it is not possible to introduce the same number of female and male organisms as larvae into each beaker, the emergence rates of male and female numbers are pooled for the statistical analysis. The probit analysis for the number of emerged midges was calculated. The day of first emergence was postponed for about one day at the test concentration 10 mg pure metabolite/l and for two days at 32 mg pure metabolite/l. The mean development time and rate were calculated for each beaker. Only at the nominal concentration of 32 mg pure metabolite/l the mean development time was 15.7% higher compared to the control. Abnormal behaviour of larvae, pupae or midges was not observed throughout the study. The influence on the development after 28 days (EC_5, EC_{10}, EC_{15} and EC_{50}) is shown in table A7_4_1_2-6.
5.3	**Conclusion**	The test is performed according to the BBA-Guideline "Effects of plant protection products on the development of sediment-dwelling larvae of *Chironomus riparius* in a water-sediment system" (1995) and fulfils the requirements.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

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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 with the following comments: **3.4.4** Test conditions, wide variation in pH (5.0 – 7.9), pH decreased during the study in all vessels except the highest concentration, likely to be due to algal growth and increased excretion of growing larvae.
Results and discussion	Accept applicant's version with the following comments: **4.2.2** On day 28, the concentration of DMSA in overlying water was between 79 and 84 %. The UK CA considers this reasonable for a 28 d static test. **4.3** The % emergence in the control was 81 % which is less than the percentage emergence in some of the test concentrations.
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	*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	Test and breeding water were prepared as "M7-medium". The medium is prepared using deionised water and adding mineral salts and vitamins. The concentrations of them in the water are given in table 1 of the report.
Alkalinity	53.4 mg/l $CaCO_3$
Hardness	196 mg/l $CaCO_3$
pH	8.1
Ca / Mg ratio	-
Na / K ratio	-
Oxygen content	9.6 mg/l
Conductance	550 µS/cm
Holding water different from dilution water	No

Table A7_4_1_2-1a: Test sediment

Sediment characterisation	Details
Particle size distribution (USDA-Norm)	Sand: 78.1% Silt: 9.3% Clay: 12.6%
Organic carbon (%)	54.5
Water holding capacity (g H_2O/100 g d.wt.s *)	83.5
pH	6.0
Cation exchange capacity (meq/100 g sediment)	10.0

* d.wt.s. = dry weight sediment

Table A7_4_1_2-2: Test organisms

Criteria	Details
Strain	*Chironomus riparius*, animals of the first larval stage (L1) were used
Source	Test specimen of *Chironomus riparius* were obtained from a culture maintained at the University of Sheffield (UK) in autumn 1991 and kept in an in-house laboratory since then.
Age (at start of the study)	1st instars < 2-3 days old
Breeding method	2-4 egg masses are placed into the prepared basin. The hatched larvae are fed with green algae and an aqueous suspension of a vegetable fish food (TetraPhyll®). After 2-3 weeks the adults emerge. After mating, female adults will lay egg masses on the water surface where these can be taken to start a new culture or to perform toxicity tests. The L1 larvae used in the study were obtained by introducing some fresh egg masses in small dishes with culture medium. Two to three days after hatching, the L1 larvae were transferred with a blunt pipette to the test vessel.
Kind of food	During the study the test organisms were fed with a commercial fish food extract (TetraPhyll®) (aqueous suspension, 1 g TetraPhyll®/20 ml water) as used for the breeding.
Amount of food	About 1 mg TetraPhyll®/larvae/day
Feeding frequency	During the study the larvae were fed at least three times per week.
Pretreatment	One day prior to treatment (day –1) the test organisms were transferred in a randomised procedure into the test containers.
Feeding of animals during test	Yes, The amount of the suspension was added to each of the test container on days: -1, 0, 3, 4, 5, 6, 7, 10, 11, 12, 13, 14, 17, 18, 19, 20, 21, 24, 25, 26 and 27

Table A7_4_1_2-3: Test system

Criteria	Details
Static test	The bottom of the test containers were covered with a 2 cm high layer of sediment. 2.65 l water were slowly poured into the beakers. The height of the water was 20 cm.
Volume of test vessels	3 l glass beakers with an average diameter of about 13.5 cm
Volume water/animal	106 ml
Number of animals/vessel	25
Number of vessels/ concentration	1-2 replicates for each test substance concentration, 3 replicates were prepared for the control
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 ± 2 °C
Dissolved oxygen	Control: 9.1 (minimum at the end) – 9.8 (maximum at day –1); Test concentrations: 9.1 (minimum at the end) - 9.9 (maximum at day –1)
pH	Control: 5.1 (minimum at the end) – 7.8 (day –1); Test concentrations: 5.0 (minimum at the end) – 7.9 (maximum at day –1); The pH-values decreased during the study in all test beakers of the control and the treatments except of the highest test concentration.
Adjustment of pH	No
Aeration of dilution water	Yes, the water was aerated and temperated to 20 °C in an in-house preparation tank. (The aeration was stopped for 24-hours after insertion of the test organism). Gentle aeration in the test containers was provided through a glass Pasteur pipette situated about 2.5 cm above the sediment layer.
Quality/Intensity of irradiation	Light intensity was on average about 2000 lux
Photoperiod	16:8 light-dark-cycle with a 30 minutes dusk and dawn period.

Table A7_4_1_2-5: Effect data

Summary of numbers of emerged midges over 28 days					
Nominal conc. (mg pure metabolite/l	No. of inserted larvae	No. of emerged midges	Emergence (%) of inserted larvae	% male of emergence	% female of emergence
Control	75	61	81	41	59
0.010	25	20	80	40	60
0.032	25	19	76	37	63
0.10	50	38	76	37	63
0.32	50	39	78	46	54
1.0	50	39	78	38	62
3.2	50	43	86	44	56
10	50	37	74	41	59
32	25	18	72	56	44
100	25	0	-	-	-

Table A7_4_1_2-6: Influence on the development after 28 days (based on nominal concentrations)

	EC_{15} (mg pure metabolite/l)	95 % c.l. (mg pure metabolite/l)	EC_5 (mg pure metabolite/l)	EC_{10} (mg pure metabolite/l)	EC_{50} (mg pure metabolite/l)
Emergence rate (pooled sex)	31.1	23.5 - 41.1	24.4	28.2	46.9
Development rate (pooled sex)	16.7	not calculable	9.7	13.4	42.3
Development rate (male)	13.8	not calculable	7.4	10.7	40.6
Development rate (female)	17.2	not calculable	10.1	13.9	43.1

The day of first emergence was postponed for about one day at the test concentration 10 mg pure metabolite/l and for two days at 32 mg pure metabolite/l.

Table A7_4_1_2-7: Analytical results of dimethylaminosulfanilid

Nominal conc. (mg pure metabolite/l)	Analytical results of dimethylaminosulfanilid, means of two analyses each (mg pure metabolite/l)					
	1 hour/day 0		day 7		day 28	
	Conc.	% of nominal conc.	Conc.	% of nominal conc.	Conc.	% of nominal conc.
	Overlying water					
Control	< 0.01	-	n.a.	-	n.a.	-
0.10	0.09	90	0.09	90	0.08	80
1.0	0.94	94	0.92	92	0.79	79
10	9.7	97	9.1	91	8.4	84
	Pore water					
Control	< 0.01	-	n.a.	-	n.a.	-
0.10	0.01	0.58	0.06	3.31	0.08	4.74
1.0	0.11	0.57	0.55	3.26	0.68	4.05
10	1.1	0.68	5.8	3.33	7.4	4.36

n.a.: not analysed