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*						
	Number		Percentage		Oxygen [mg/l]	pH	Temperature [°C]
	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 ≥ 80% of initial concentration during test	X	

Criteria for poorly soluble test substances	-	-

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**	[redacted] 1989, Toxicity of Euparen WG 50 to *Scenedesmus Subspicatus* (OECD – Algae Growth Inhibition Test) [redacted]	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
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: 233715493	
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.

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA VII.7.3 *Scenedesmus Subspicatus*

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	[redacted]
Results and discussion	[redacted]
Conclusion **Reliability** **Acceptability** **Remarks**	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA VII.7.3 *Scenedesmus Subspicatus*

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	[redacted]
Laboratory culture	Yes
Method of cultivation	Standardised conditions
Pretreatment	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	-	-

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**	[redacted] 1997, Dimethylaminosulfanilid (DMSA) Alga, Growth Inhibition Test [redacted]	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
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	Article number: 00436151	
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	-	

Section A7.4.1.3 Growth inhibition test on algae

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

Scenedesmus subspicatus

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

Section A7.4.1.3 Growth inhibition test on algae

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

Scenedesmus subspicatus

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 [redacted]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	
Reliability	
Acceptability	
Remarks	
	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*

Section A7.4.1.3 Growth inhibition test on algae

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

Scenedesmus subspicatus

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	[redacted]
Laboratory culture	Yes
Method of cultivation	Cultivation of stock cultures, pre - cultures and test cultures took place in a light chamber at 23 ± 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 ± 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 µE/m² × 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	-	-

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA VII.7.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] 2001, Preventol A 4-S Toxicity to Bacteria [redacted]	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
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 Commission Directive 88/302/EEC, Part C. This test method is in most parts identical with OECD guideline No. 209	
2.2	**GLP**	Yes	
2.3	**Deviations**	No, the study is comparable to OECD guideline No. 209	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2 of dossier	
3.1.1	Lot/Batch number	Batch number: 233014092	
3.1.2	Specification	As given in Section 2 of dossier	
3.1.3	Purity	[redacted]	X
3.1.4	Composition of Product	-	
3.1.5	Further relevant properties	-	
3.1.6	Method of analysis	Test substance concentrations are not confirmed by analytical method	X
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Type of application of the test substance: direct weighing. The test substance has been added to about 130 ml deionised water and stirred overnight before testing (equilibration phase).	
3.3	**Reference substance**	Yes, 3,5-Dichlorophenol	
3.3.1	Method of analysis for reference substance	Reference substance concentrations are not confirmed by analytical method	X

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA VII.7.4

3.4	**Testing procedure**		
3.4.1	Culture medium	Synthetic medium	X
3.4.2	Inoculum / test organism	see table A7_4_1_4-1	
3.4.3	Test system	No data	X
3.4.4	Test conditions	see table A7_4_1_4-2	
3.4.5	Duration of the test	3 hours with permanent aeration	
3.4.6	Test parameter	Respiration inhibition	
3.4.7	Analytical parameter	Oxygen measurement	
3.4.8	Sampling	The oxygen concentration was measured in the controls and in every concentration of the test and reference substance at the beginning and at the end of the test period. pH and temperature were determined in the controls and in every test concentration of test and reference substance during the test period.	
3.4.9	Monitoring of TS concentration	No	
3.4.10	Controls	Two controls without test substance are included in the test design, one at the start and the other at the end of the test series. A physico - chemical oxygen consumption control with a test substance concentration of 10000 mg/l was carried out, since some substances can also consume oxygen by chemical reactivity.	
3.4.11	Statistics	An EC_{50} value is calculated from determinations at different concentrations using statistical methods (probit analysis).	
		4 RESULTS	
4.1	**Preliminary test**	Not performed	
4.1.1	Concentration	-	
4.1.2	Effect data	-	
4.2	**Results test substance**		
4.2.1	Initial concentration of test substance	Nominal concentrations: 5.6, 10, 18, 32 and 56 mg/l	
4.2.2	Actual concentrations of test substance	The test substance concentrations are not confirmed by analytical methods	
4.2.3	Growth curves	No graph available	
4.2.4	Cell concentration data	Not reported	

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA VII.7.4

4.2.5	Concentration/ response curve	Concentration/response curves (inhibition vs. concentration) are given in the report on page 17 (test substance) and on page 20 (reference substance)
4.2.6	Effect data	EC_{50} = 19 mg/l
4.2.7	Other observed effects	-
4.3	**Results of controls**	No physico – chemical oxygen consumption has been determined at 10000 mg/l test substance concentration.
4.4	**Test with reference substance**	Performed with 3,5-Dichlorophenol
4.4.1	Concentrations	2.5, 5, 10, 20 and 40 mg/l
4.4.2	Results	EC_{50} = 12 mg/l

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	To assess the toxicity of dichlofluanid to bacteria a test was investigated according to the Commission Directive 88/302/EEC, Part C. This method is in most parts identical with OECD guideline No. 209. Activated sludge was exposed to dichlofluanid at different concentrations. The respiration rate of each mixture was determined after aeration periods of 3 hours. The test shows no significant deviations from the OECD guideline No. 209.
5.2	**Results and discussion**	50% inhibition of respiration was determined at EC_{50} = 19 mg/l dichlofluanid. No physico – chemical oxygen consumption has been determined at 10000 mg/l test substance concentration. Therefore lower concentrations of the test substance cause no physico – chemical oxygen consumption (deduced values). At nominal test concentrations of 5.6 – 56 mg/l, inhibition of respiration in activated sludge was observed between 23.4% and 76.4% (see table A7_4_1_4-3)
5.2.1	EC_{20}	-
5.2.2	EC_{50}	19 mg/l
5.2.3	EC_{80}	-
5.3	**Conclusion**	All validity criteria of the test method were met: * respiratory rate of the two controls differs less than 15% * respiratory rate of the controls is < 60 mg O_2/l·h * EC_{50} of the reference substance 3.5-Dichlorophenol is in the range 5–30 mg/l A dose – response relationship can be seen from the test.

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA VII.7.4

5.3.1	Reliability	2
5.3.2	Deficiencies	Yes, Some reporting deficiencies: No data about the test system. Information incomplete about culture medium and test organism.

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA VII.7.4

	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	[redacted]
Results and discussion	[redacted]
Conclusion	
Reliability	
Acceptability	
Remarks	
	[redacted]
	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_4-1: Inoculum/Test organism

Criteria	Details
Nature	activated sludge (mixed population of aquatic micro-organisms)
Species	-
Strain	-
Source	Waste water treatment plant treating predominantly domestic sewage
Sampling site	Aeration tank of the waste water treatment plant (Wupper area water authority)
Laboratory culture	Yes
Method of cultivation	-
Preparation of inoculum for exposure	No data
Pretreatment	Aeration of the activated sludge; daily feed with synthetic medium
Initial cell concentration	Only 280 mg suspended solids/l were used because of strong respiration of the activated sludge

Table A7_4_1_4-2: Test conditions

Criteria	Details
Test temperature	18.6 – 19.3 °C
pH	8.1 – 8.3 7.6 (physico chemical oxygen consumption control)
Aeration of dilution water	No data
Suspended solids concentration	280 mg/l

Table A7_4_1_4-3: Test results of test substance (based on nominal concentrations) and controls

Test Compound [mg/l]	Respiratory Rate [mg O_2/l h]	Inhibition [%]
5.6	24.8	23.9
10	24.0	26.4
18	15.4	52.8
32	12.0	63.2
56	7.7	76.4

Control	Respiratory rate [mg O_2/l·h]
Control 1	33.6
Control 2	31.5

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA VII.7.4 **DIMETHYLAMINOSULFANILID (DMSA)**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] 1998, DMSA Toxicity to Bacteria [redacted] amended 2002-09-03	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
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 Commission Directive 88/302/EEC, Part C. This test method is in most parts identical with OECD guideline No. 209	
2.2	**GLP**	Yes	
2.3	**Deviations**	No, the study is comparable to OECD guideline No. 209	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Dimethylaminosulfanilid (DMSA)	
3.1.1	Lot/Batch number	No data	X
3.1.2	Specification		
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	The test substance concentrations are not confirmed by analytical method	X
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Type of application of test substance: direct weighing	
3.3	**Reference substance**	Yes 3,5-Dichlorophenol	
3.3.1	Method of analysis for reference substance	The reference substance concentrations are not confirmed by analytical method	X

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA VII.7.4 **DIMETHYLAMINOSULFANILID (DMSA)**

3.4	**Testing procedure**		
3.4.1	Culture medium	No data	X
3.4.2	Inoculum / test organism	Activated sludge (mixed population of different micro-organisms) from a laboratory scale sewage treatment unit which runs predominantly with domestic sewage (South Wupper area water authority). Without pre – treatment.	
3.4.3	Test system	No data	X
3.4.4	Test conditions	see table A7_4_1_4-1	
3.4.5	Duration of the test	30 minutes with permanent aeration	
3.4.6	Test parameter	Respiration inhibition	
3.4.7	Analytical parameter	Oxygen measurement	
3.4.8	Sampling	The oxygen concentration was measured in the controls and in every concentration of the test and reference substance at the beginning and at the end of the test period. pH and temperature were determined in the controls and in every test concentration of test and reference substance during the test period.	
3.4.9	Monitoring of TS concentration	No	
3.4.10	Controls	Two controls without test substance are included in the test design, one at the start and the other at the end of the test series. A physico - chemical oxygen consumption control with a test substance concentration of 10000 mg/l was carried out, since some substances can also consume oxygen by chemical reactivity.	
3.4.11	Statistics	An EC_{50} value was calculated from determinations at different concentrations (probit analysis was used for the reference substance).	X
		4 RESULTS	
4.1	**Preliminary test**	Not performed	
4.1.1	Concentration	-	
4.1.2	Effect data	-	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	Nominal concentrations: 320, 560, 1000, 1800 and 3200 mg/l	
4.2.2	Actual concentrations of test substance	The test substance concentrations are not confirmed by analytical method.	
4.2.3	Growth curves	No graph available	

4.2.4	Cell concentration data	Not reported
4.2.5	Concentration/ response curve	Concentration/response curves (inhibition vs. concentration) are given in the report on page 17 (test substance) and on page 19 (reference substance)
4.2.6	Effect data	EC_{50} = 1140 mg/l
4.2.7	Other observed effects	-
4.3	**Results of controls**	No physico – chemical oxygen consumption has been determined at 10000 mg/l test substance concentration.
4.4	**Test with reference substance**	Performed with 3,5-Dichlorophenol
4.4.1	Concentrations	2.5, 5, 10, 20 and 40 mg/l
4.4.2	Results	EC_{50} = 14 mg/l

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	To assess the toxicity of dimethylaminosulfanilid (DMSA) to bacteria a test was conducted according to the Commission Directive 88/302/EEC, Part C. This method is in most parts identical with OECD guideline No. 209. Activated sludge was exposed to dimethylaminosulfanilid at different concentrations. The respiration rate of each mixture was determined after aeration periods of 30 minutes. Comparison with the OECD guideline No. 209 shows no significant deviations.
5.2	**Results and discussion**	50% inhibition of respiration was determined at EC_{50} = 1140 mg/l dimethylaminosulfanilid. No physico – chemical oxygen consumption has been determined at 10000 mg/l test substance concentration. Therefore lower concentrations of the test substance cause no physico – chemical oxygen consumption (deduced values). At nominal test concentrations of 320 – 3200 mg/l, inhibition of respiration in activated sludge was observed between 29.4% and 63.6% (see table A7_4_1_4-2)
5.2.1	EC_{20}	-
5.2.2	EC_{50}	1140 mg/l
5.2.3	EC_{80}	-
5.3	**Conclusion**	All validity criteria of the test method were met: * respiratory rate of the two controls differs less than 15% * respiratory rate of the controls is < 60 mg O_2/l·h * EC_{50} of the reference substance 3.5-Dichlorophenol is in the range

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA VII.7.4 **DIMETHYLAMINOSULFANILID (DMSA)**

		5–30 mg/l
		A dose – response relationship can be seen from the test.
5.3.1	Reliability	2
5.3.2	Deficiencies	Yes,
		Some reporting deficiencies:
		No lot or batch no. mentioned.
		No data about the composition of the culture medium and the test system.
		Method used for the calculation of EC_{50} value not mentioned for the test substance

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA VII.7.4 **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	28/01/05
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	
Reliability	
Acceptability	
Remarks	
	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*

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA VII.7.4 **DIMETHYLAMINOSULFANILID (DMSA)**

Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_4_1_4-1 Test conditions

Criteria	Details
Test temperature	19.7 – 20.0 °C
pH	7.8 – 7.9 7.2 (physico chemical oxygen consumption control)
Aeration of dilution water	No data
Suspended solids concentration	Only 400 mg/l were used because of strong respiration of the activated sludge

Table A7_4_1_4-2: Test results of test substance (based on nominal concentrations)

Test concentration [mg/l]	Respiratory Rate [mg O_2/l·h]	Inhibition [%]
320	23.3	29.4
560	20.0	39.4
1000	16.8	49.1
1800	13.5	59.1
3200	12.0	63.6

The respiratory rate was determined to be 33.0 mg O_2/l·h for each of the two controls

Section A7.4.2 Bioconcentration in aquatic organisms (fish)

Annex Point IIA, VII.7.5 *Lepomis macrochirus*

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] 1991, Dichlofluanid: Bioconcentration in fish [redacted]
1.2	**Data protection**	Yes
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
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, EPA guideline72-6, 165-4 (1982)
2.2	**GLP**	Yes
2.3	**Deviations**	No, with regard to OECD guideline No. 305

3 MATERIALS AND METHODS

3.1	**Test material**	[Ring-UL-^{14}C]-dichlofluanid, specific radioactivity: 370 kBq/mg (10 µCi/mg)
3.1.1	Lot/Batch number	Batch No.: Lager Nr. 9507
3.1.2	Specification	[Ring-UL-^{14}C]-dichlofluanid
3.1.3	Purity	[redacted]
3.1.4	Further relevant properties	Water solubility (a.i.): 1.3 mg/l
3.1.5	Radiolabelling	[Ring-UL-^{14}C]-dichlofluanid
3.1.6	Method of analysis	Samples were analysed in accordance with the following conditions. <u>Processing of fish samples for radioassay</u> Fish samples were collected and dissected into edible (body, muscle, skin, skeleton, fins) and non-edible (head, internal organs) portions. Samples were transferred into weighed polystyrene vials suitable for further handling. After determining the wet weight of the samples they were lyophilized, reweighed and homogenized. Aliquotal parts were taken for radioactivity measurement. <u>Radioactivity measurement</u> Normally triplicate sub samples were analysed due to inhomogeneities in the freeze-dried tissues caused mainly by the scales. The radioactivity of extracts and solutions was determined by means of liquid scintillation measurement (LS-measurement); solid samples were combusted before the LS-measurement.
3.2	**Reference substance**	No

3.2.1	Method of analysis for reference substance	-	
3.3	**Testing/estimation procedure**		
3.3.1	Test system/ performance	Test animals The bluegill sunfish (*Lepomis macrochirus*) (lot 3/90) used in this 42-day dynamic study were obtained [redacted] The fish were identified to species by the supplier. All test fish were held in culture tanks on a 16-hour daylight photoperiod and observed for at least 14 days prior to testing. [redacted] During the acclimation and test periods, the fish received a Kronen-Fisch, Typ I (Rheinkrone, D-4230 Wesel) fish food once daily ad libitum. Upon arrival a prophylactic treatment with oxytetracycline for fin rot disease was performed. No mortality occurred in the fish lot used 14 days prior to the introduction of the fish in the test medium. Test system A dosing system comprising a Hamilton [R]Microlab MT dispenser with a 250 µl-syringe for each aquarium controlled by an EPSON HX20 computer (for dosing of stock solution) and flow-meters (for water flow control) was used for the introduction of [^{14}C]-dichlofluanid and diluent water into the 100 litre test aquaria. Aerated reconstituted water was delivered to the glass aquaria at an average rate of approx. 25 l per hour per aquarium during the exposure period (28 days), an amount sufficient to replace the approximately 100 litre test volume about 6 times in a 24-hour period and stock solution ([^{14}C]-dichlofluanid in acetone) was dosed at a rate of 50 µl every 72 seconds (= 2.5 ml/h). Water (continuously 25 l/h) and aliquots of [^{14}C]-dichlofluanid stock solution (50 mg/l, 0.05 ml every 72 sec) were delivered to a 2000 ml-mixing cell to yield a nominal exposure concentration of 5.0 µg/l. The mixture was running continuously from the mixing vessel into the respective aquarium. The control aquarium received an amount of acetone solvent (0.1 ml/l) as the exposure aquarium. The exposure system consisted of one 5.0 µg/l nominal concentration aquarium and one control aquarium. The aquaria were labelled with the study number and treatment level. The test aquaria arranged in a lab room were kept at 22 °C (± 1) by adding diluent water electronically thermostated to that temperature. Temperature was measured once daily on working days and the range of temperature deviations was followed by a mercury-minimum-maximum-thermometer, which was reset after each reading. The diluter system was calibrated by volumetric measurements of syringe dispenser aliquots and flow-rate of flow meters. Preparation of the test substance The stock solution for the treatment of the fish were prepared as follows:	X

The radioactive material (ca. 37 MBq, 370 kBq/mg delivered in a 2 l bottle) was diluted in 2 l acetone p.a. by adding the solvent to the tracer. Thus the concentration in the stock solution was 50 mg/l with a specific radioactivity of 370 kBq/mg or 22200 dpm/µg (equivalent to 777 dpm per 7 ml-sample of test medium).

Test procedure

Uptake phase

The uptake phase was initiated by transferring groups of 56 randomly selected and previously acclimated fish (length 6.66 ± 0.66 cm) to the test chamber. The initial loading was 2.3 g fish/l and 0.38 g fish/l/day. The fish were observed initially and every 24 hours on working days thereafter during the exposure period of 28 days for mortality and/or adverse behaviour. At the same intervals pH and dissolved oxygen were measured in all aquaria. The temperature was recorded hourly in the control tank. Water and fish were sampled throughout the uptake period on day 0, 1, 3, 8, 10, 14, 21 and 28. The water and fish samples were radioassayed and analysed according to the indicated conditions.

Depuration phase

On day 28 of the exposure period, the addition of the [^{14}C]-dichlofluanid test material ceased. At the beginning of the depuration phase, the aquaria were cleaned mechanically, emptied by suction to a water height of ca. 5 cm, and filled with uncontaminated diluent water (22 °C). During that procedure the fish remained in the aquaria. The fish were then exposed to flowing uncontaminated diluent water (22 °C) for 14 days. During the depuration period, water and fish were sampled on day 29, 31, 35, 38 and 42. The samples were radioassayed according to the indicated conditions. The fish were observed initially and every 24 hours on working days during the depuration period of 14 days for mortality and/or adverse behaviour. At the same intervals pH and dissolved oxygen were measured in all aquaria. The temperature was recorded hourly in the control tank.

Sampling

Fish

Fish were sampled on day 0, 1, 3, 8, 10, 14, 21, 28, 29, 31, 35, 38 and 42. On these dates, four fish from each chamber were collected and processed individually. The fish were dissected into edible and viscera/non-edible parts. Samples were treated and measured according to the indicated conditions. Fresh weight and dry weight of the fish portions were determined.

Water

At each sampling day, 3 samples of 7 ml of water were removed from each aquarium. The concentrations of ^{14}C calculated as [^{14}C]-dichlofluanid in water were calculated by liquid scintillation counting of triplicate 7 ml-samples pipetted directly from each control and test tank. 7 ml scintillation cocktail were added to each sample.

Chemical and physical test parameters

Water quality parameters of dissolved oxygen and pH were measured

initially and throughout the study at least on working days in the control and exposure chambers. The temperature was recorded hourly. The test chambers were not aerated throughout the test. Dissolved oxygen levels remained at or above 96% saturation.

3.3.2 Estimation of bioconcentration

Calculation of results

In evaluating the data obtained from the bioconcentration study, a steady-state approach was used. This consists of a two compartment model (water and fish) which is used to describe the movement of the test material in and out of the test fish. This approach is used to determine the steady-state bioconcentration factor (BCF), the uptake rate constant and the depuration rate constant.

The water concentration of dichlofluanid was calculated by the formula:

Net dpm/ml = Activity of test water – Activity of control water (dpm/ml)

$$\text{Water concentration } \mu g/l = \frac{(\text{net dpm/ml}) * 1000}{\text{specific activity of parent compound (dpm/}\mu g)}$$

The tissue concentration of dichlofluanid was calculated by the formula:

Net dpm/g = Activity of test tissue – Activity of control tissue (dpm/g)

$$\text{Tissue concentration mg/kg} = \frac{(\text{net dpm/g}) * 1000}{\text{specific activity of parent compound (dpm/mg)}}$$

Bioconcentration factors for fish portions were determined by dividing the [^{14}C]-tissue radioactivity by the mean [^{14}C]-water radioactivity up to and including that day.

dpm/ml (water) = (dpm (treated) - dpm (control))/sample volume

Sample volume in all water radioactivity measurements was 7 ml.

$$\text{dpm/g (fish portion)} = \frac{(\text{dpm/g dry weight (treated) - dpm/g dry weight (control)})}{(\text{sample weight (fresh) / sample weight (dry)})}$$

$$\text{Bioconcentration factor} = \frac{\text{dpm/g (fish portion)}}{\text{dpm/ml (water)}}$$

Bioconcentration factor for whole fish were determined by the following calculation:

$$BCF(T) = \frac{(BCF(E) * \text{fresh weight}(E)) + (BCF(V) * \text{fresh weight}(V))}{(\text{fresh weight}(E) + \text{fresh weight}(V))}$$

(T) = whole fish
(E) = edible portion
(V) = non-edible portion

Statistics

The uptake rate constant and depuration rate constant were determined by the Dow BIOFAC computer program. The BCF at steady state, the time to reach 90% of steady state and the time to reach 1/2 of test compound clearance (depuration) were also calculated from the estimated rate constants. A measure of the variability of the estimated parameters was provided by the standard deviation of each estimate. The measured bioconcentration factors in the fish samples were multiplied with the mean exposure concentration of 4.4 µg/l.

4 RESULTS

4.1 Experimental data

4.1.1	Mortality/behaviour	Fish showed no abnormal behaviour throughout the test.
4.1.2	Lipid content	Lipid content was not determined
4.1.3	Concentrations of test material during test	Individual values of radioactivity and concentration of the test substance in test water are given in the report (table 2). Water concentrations ranged from 4.1 µg/l to 4.7 µg/l through 28 days of the bioconcentration (uptake) phase. The average water concentration (using the mean value for each sample) during the uptake phase was 4.4 (± 0.2) µg/l. Results of radioanalysis (individual and mean values) of [^{14}C]-dichlofluanid for all sampling times in edible tissue and non-edible tissue during 28 days of constant exposure to [^{14}C]-dichlofluanid and 14 days of depuration in clean water are summarised in table 9-14 of the report. A graph showing the uptake and depuration of the test material in the test organism (whole fish) and the time to steady-state is given in figure 3 of the report. The mean tissue residues at steady state were calculated to be 0.27 mg/kg for edible tissue, 0.38 mg/kg for viscera and 0.32 mg/kg for whole fish. The depuration half-lives are provided in table A7_4_2-1.
4.1.4	Bioconcentration factor (BCF)	The bioconcentration factors of the whole fish and the edible parts are 72 (± 14) and 61 (± 9), respectively. The BIOFAC calculated BCF values for whole fish and edible parts corresponded well with the respective average steady-state bioconcentration factors of 73 X and 62 X for [^{14}C]-dichlofluanid for days 8, 10, 14, 21 and 28. Time to reach 90% of steady state in the whole fish was 0.8 days.

Section A7.4.2 Bioconcentration in aquatic organisms (fish)

Annex Point IIA, VII.7.5 *Lepomis macrochirus*

4.1.5	Uptake and depuration rate constants	See table A7_4_2-1
4.1.6	Depuration time	See table A7_4_2-1
4.1.7	Metabolites	No metabolites identified
4.1.8	Other Observations	-
4.2	**Estimation of bioconcentration**	Bioconcentration factor is based on measurements.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

A dynamic 42-day study (28 days of exposure, 14 days for the depuration part) was conducted according to EPA guideline 72-6, 165-4 (1982) to evaluate the bioconcentration of [^{14}C]-dichlofluanid by bluegill sunfish (*Lepomis macrochirus*). A computer-controlled dosing system allowed approximately 6 renewals of each 100 l aquaria in a 24 hour-period. The test fish were exposed to one solvent control and one treated group (nominal concentration: 5.0 µg/l). Acetone (0.1 ml/l) was used as solvent.
Radioanalysis (^{14}C-CO_2) of edible and non-edible portions of individual fish was performed at different time points throughout the exposure and depuration period. At the same time points radioactivity in water samples was measured. Bioconcentration factors (BCF) were calculated for total [^{14}C]-residues. BCFs for whole fish were calculated from BCFs of edible and non-edible portions. The kinetic data were calculated using a computer program (BIOFAC).

The study shows no significant deviations from test guideline.

5.2 Results and discussion

Fish showed no abnormal behaviour throughout the test.

Temperature remained at 22 °C; the dissolved oxygen concentrations ranged between 7.3 and 9.6 mg/l, corresponding to 83 – 109% saturation at the respective temperature and were considered adequate for testing; the pH values of the treated chamber were consistent with the control throughout the study and ranged from 6.9 to 7.7.

Results of the study (calculated with BIOFAC) are given in table A7_4_2-1.

The bioconcentration factors of the whole fish and the edible parts are 72 (± 14) and 61 (± 9), respectively.
The BIOFAC calculated BCF values for edible parts and whole fish corresponded well with the respective average steady-state bioconcentration factors of 62 X and 73 X for [^{14}C]-dichlofluanid for days 8, 10, 14, 21 and 28. These values correspond to calculated steady-state total residue levels of 0.27 and 0.32 mg [14C]-dichlofluanid equivalents/kg for edible parts and whole fish, respectively.

Time to reach 90% of steady state in the whole fish was 0.8 days.

24 hours after cessation of exposure 84, 86 and 85% of the maximum measured plateau residues were depurated from edible portions, non-edible portions and whole fish, respectively. After seven days in uncontaminated water more than 99% of the maximum plateau radio-activity was depurated from edible portions, non-edible portions and whole fish, respectively.

The half life for clearance from whole fish was 0.24 days.

5.3 Conclusion

Validity criteria can be considered as fulfilled.

Dichlofluanid is accumulated very rapidly by bluegill sunfish with a total residue bioconcentration factor of 73 X for whole fish. When exposure ceases, the residues are depurated quickly with a half-life of less than 6 hours. Accumulation in edible parts is less (62 X) than in whole fish (73 X).

Time to reach 90% of steady state in the whole fish was 0.8 days.

Section A7.4.2 Bioconcentration in aquatic organisms (fish)

Annex Point IIA, VII.7.5 *Lepomis macrochirus*

		The bioconcentration factor for dichlofluanid may be overestimated in this study because all calculations refer to radioactivity. Thus the BCFs given in this report refer to total residues from exposure to a constant concentration of dichlofluanid.
5.3.1	Reliability	1
5.3.2	Deficiencies	No, with regard to the OECD guideline No. 305 but: Study was performed with one concentration of the test substance instead of at least two; No replicates

Section A7.4.2 Bioconcentration in aquatic organisms (fish)

Annex Point IIA, VII.7.5 *Lepomis macrochirus*

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	[redacted]
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	
	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*
Reliability	*Discuss if deviating from view of rapporteur member state*
Findings	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_4_2-1: Results of the study (calculated with BIOFAC)

	edible	whole fish	viscera
Bioconcentration factor (BCF)	61 (±9)	72 (±14)	87 (±13)
Time to reach 90% of Steady-State, [days]	0.82 (±0.09)	0.80 (±0.11)	1.27 (±0.14)
t(1/2) for clearance, [days]	0.25 (±0.03)	0.24 (±0.03)	0.38 (±0.04)
Uptake rate constant, [1/day]	172 (±18)	209 (±28)	156 (±17)
Clearance rate constant, [1/day]	2.8 (±0.3)	2.9 (±0.4)	1.8 (±0.2)

Section A7.4.3.1 Prolonged toxicity to an appropriate species of fish

Annex Point IIIA XIII.2.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] 1989, Toxicity of Dichlofluanide techn. (VM 90) for Rainbow Trout (*Salmo gairdneri*) with prolonged exposure (21 days) [redacted]	
		An amendment of the report is currently (March 2004) under preparation.	X
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
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**	Dichlofluanid techn. [redacted] premixture = VM [redacted]	
3.1.1	Lot/Batch number	Lot number: 1530 B after 00670/1146 B	
3.1.2	Specification	[redacted] lichlofluanid	
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	-	
3.1.5	Further relevant properties	-	
3.1.6	Method of analysis	No method mentioned used for the determination of the test substance concentrations in the test medium	X
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	see table A7_4_3_1-1	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	-	

Section A7.4.3.1 Prolonged toxicity to an appropriate species of fish

Annex Point IIIA XIII.2.1

3.4	**Testing procedure**	
3.4.1	Dilution water	See table A7_4_3_1-2
3.4.2	Test organisms	See table A7_4_3_1-3
3.4.3	Test system	See table A7_4_3_1-4
3.4.4	Test conditions	See table A7_4_3_1-5
3.4.5	Duration of the test	21 days
3.4.6	Test parameter	Mortality and observable symptoms
3.4.7	Sampling	The fish were examined for symptoms of intoxication and mortality every working day but at least three times per week (each test level, control and acetone solvent control). The oxygen concentration and the pH were measured every working day in each aquarium. The temperature was measured continuously in one aquarium and recorded hourly. Body weight and length of the fish were measured for a representative random sample of the test population of fish before the start and for all surviving fish at the end of the experiment.
3.4.8	Monitoring of TS concentration	Yes The concentrations of active substance and the decomposition product DMSA in the test medium were determined at the start and end of the experiment. Concentrations below the NOEC were not analysed.
3.4.9	Statistics	Statistical analysis of results for a 21 – day LC_{50} value and its corresponding 95% confidence limit was calculated using the method of sliding means. 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.
		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: 2.97, 6.40, 13.78, 29.70 and 63.98 µg a.i./l,

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		corresponding to 3.25, 7.00, 15.08, 32.49 and 70.00 µg test substance /l	
4.2.2	Actual concentrations of test substance	Despite the technical problems that arise in testing such hydrolytically instable compounds even under flow-through conditions, it was demonstrated by analyses that exposure concentrations were kept constant within a narrow range. Accordingly the results of this study can be recalculated based on the mean measured concentrations. Measured concentrations of dichlofluanid at the various time points are given in table A7_4_3_1-8. Actual concentrations of DMSA are given in table A7_4_3_1-9	X
4.2.3	Effect data (Mortality)	See table A7_4_3_1-6 and table A7_4_3_1-7	
4.2.4	Concentration / response curve	The mortality increases from 10% to 100% between doses of 13.78 and (10% mortality) and 29.70 µg/l (100% mortality). The presentation of a concentration/response curve is therefore not useful.	X
4.2.5	Other effects	Observable symptoms were noted among the fish in the 13.78 and 29.70 µg/l test levels. The body weights and length of the surviving fish at the end of the exposure time do not differ significantly ($p \leq 0.05$) from those of the controls.	
4.3	**Results of controls**		
4.3.1	Number/ percentage of animals showing adverse effects	No mortality or behavioural changes occurred in the controls. In the solvent control group, one fish died from cannibalism during the experiment.	
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 dichlofluanid techn. (used as VM[redacted] for rainbow trout (*Salmo gairdneri*) with prolonged exposure was investigated in a 21 – day flow – through experiment in accordance with the OECD guideline No. 204. The test shows no significant deviations from the guideline.	
5.2	**Results and discussion**	Based on nominal values a 21 - day LC_{50} value (with 95% confidence interval) was calculated to be 18.65 (16.00 – 21.73) µg a.i./l, based on the nominal test concentrations of dichlofluanid. It was demonstrated by analyses that exposure concentrations were kept	X

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		constant within a narrow range. Accordingly the results of this study can be recalculated based on the mean measured concentrations. According to this recalculation the NOEC, LOEC and LC_{50} for 21 day exposure are 4.55, 9.3 and 12.3 µg dichlofluanid/l, respectively. It should further be noted that toxicity did not increase significantly after day 1 (LC_{50} = 15.2 µg/l) although exposure was kept constant during this flow-through study. The toxicity pattern is typical for dichlofluanid. No mortality or behavioural changes occurred in the controls. In the solvent control group, one fish died from cannibalism during the experiment. The body weights and length of the surviving fish at the end of the exposure time do not differ significantly ($p \leq 0.05$) from those of the controls.
5.2.1	21d-LC_{50}	18.65 µg a.i./l; recalculation based on measured concentrations: 12.3 µg a.i./l
5.2.2	NOEC	6.40 µg a.i./l; recalculation based on measured concentrations: 4.55 µg a.i./l
5.2.3	LOEC	13.78 µg a.i./l; recalculation based on measured concentrations: 9.3 µg a.i./l
5.3	**Conclusion**	The validity criteria are summarised in table A7_4_3_1-10. The active substance was not sufficiently stable in the test medium and the concentrations of dichlofluanid did not reach 80% of the theoretical value. The analytical data show that the active substance is degraded to DMSA. The geometric mean of the sum of dichlofluanid and DMSA at the start and end of the experiment was nevertheless above 80% of the theoretical value for dichlofluanid for all the biological relevant test concentrations (6.4 – 29.7 µg a.i./l). It is thus demonstrated that sufficient amounts of the active substance passed into the aquaria. It was demonstrated by analyses that exposure concentrations were kept constant within a narrow range. Accordingly the results of this study can be recalculated based on the mean measured concentrations. Measured concentrations of dichlofluanid at the various time points are given in table A7_4_3_1-8. A dose – response curve is not given, but it can be seen from the results that this curve must be very steep since the mortality increases from 10% to 100% between doses of 13.78 and (10% mortality) and 29.70 µg/l (100% mortality).
5.3.1	Other Conclusions	-
5.3.2	Reliability	2
5.3.3	Deficiencies	Yes, no method mentioned used for the determination of the test substance in the test medium (only reference to RA-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	30/08/05
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*

Section A7.4.3.1 Prolonged toxicity to an appropriate species of fish

Annex Point IIIA XIII.2.1

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_3_1-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes Mixing vessel
Vehicle	Yes A stock solution of the test substance in acetone was prepared for each concentration to be tested.
Concentration of vehicle	Solvent concentration in all the aquaria except the control was 100 µl/l
Vehicle control performed	Yes Observation for mortality and observable symptoms was performed
Other procedures	-

Table A7_4_3_1-2: 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 (2.8 – 3.1 °dH)
pH	7.2 (Control at day 0 of the test)
Oxygen content	10.0 mg/l (Control at day 0 of the test)
Conductance	-
Holding water different from dilution water	No

Table A7_4_3_1-3: Test organisms

Criteria	Details
Species/strain	Rainbow trout (*Salmo gairdneri*)
Source	[redacted]
Wild caught	No
Age/size	The mean body weight of the fish at the start of the test was 2.3 ± 0.6 g and the mean body length was 5.9 ± 0.5 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_1-4: Test system

Criteria	Details
Test type	Flow-through
Renewal of test solution	Test water flowed continuously into the test vessels at a rate of 10 litres per hour. The test volume was 40 litres. Accordingly the test volume was exchanged 6 times per day.
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_1-5: Test conditions

Criteria	Details
Test temperature	15 ± 2 °C
Dissolved oxygen	8.2 – 10.7 mg/l
pH	6.6 – 7.6
Adjustment of pH	No
Aeration of dilution water	Yes (pretreatment)
Intensity of irradiation	-
Photoperiod	16 hours light/8 hours dark

Table A7_4_3_1-6: Mortality data

Day no.	Mortality (number of fish)						
	Test Substance: Nominal concentration [µg/l]						
	Control	Solvent control	2.97	6.40	13.78	29.70	63.98
1	0	0	0	0	0	8	10
4	0	0	0	0	0	10	10
5	0	0	0	0	0	10	10
6	0	0	0	0	1	10	10
7	0	0	0	0	1	10	10
10	0	0	0	0	1	10	10
12	0	0	0	0	1	10	10
13	0	0	0	0	1	10	10
14	0	0	0	0	1	10	10
15	0	0	0	0	1	10	10
18	0	1 *)	0	0	1	10	10
19	0	1	0	0	1	10	10
20	0	1	0	0	1	10	10
21	0	1	0	0	1	10	10
Temperature [°C]	15 ± 2						
pH	6.6 – 7.6						
Oxygen [mg/l]	8.2 – 10.7						

*) One fish died due to cannibalism

Table A7_4_3_1-7: Effect data based on nominal concentrations

	21 d [µg a.i./l][1]	95 % c.l.
LC_{50}	18.65	16.00 – 21.73
NOEC	6.40	-
LOEC	13.78	-

Table A7_4_3_1-8: Recalculation of the results based on mean measured concentrations

	Nominal concentration of dichlofluanid (µg/l)			
	6.4	14	30	64
Sampling time	**Measured concentration of dichlofluanid (µg/l)**			
Day 0	5.0	8.6	21	46
Day 1	-	-	-	24
Day 4	-	-	17	-
Day 21	4.1	10	-	-
Mean measured concentration	**4.55 (= NOEC)**	**9.3 (= LOEC)**	**19**	**35**
Cumulated mortality	0 %	10 %	100 %	100 %

Table A7_4_3_1-9: Actual concentrations of DMSA

	6.4	14	30	64
Sampling time	**Actual concentration of DMSA (µg/l)**			
Day 0	n.d.	n.d.	n.d.	n.d.
Day 1	-	-	-	15
Day 4	-	-	9.1	-
Day 21	2.4	2.8	-	-

Table A7_4_3_1-10: 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	X	

Section A7.4.3.1 Prolonged toxicity to an appropriate species of fish

Annex Point IIIA XIII.2.1 **DIMETHYLAMINOSULFANILID (DMSA)**

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted] 1990, Toxicity of DMSA for Rainbow Trout (*Oncorhynchus Mykiss*) with prolonged exposure (21 days) [redacted]
1.2	**Data protection**	Yes
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
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: 900320 ELB 06
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

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	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). The oxygen concentration and the pH were measured every working day in each aquarium. 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.	
3.4.8	Monitoring of TS concentration	Yes, 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.	
3.4.9	Statistics	The NOEC was referenced to the most sensitive parameter (observed toxic symptoms or growth). 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.	
		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	X
4.2.3	Effect data	see table A7_4_3_1-5 and table A7_4_3_1-6	

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 (*Oncorhynchus mykiss*) 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	[redacted]
Results and discussion	
Conclusion	[redacted]
Reliability	
Acceptability	
Remarks	
	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_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 (*Oncorhynchus mykiss*)
Source	[redacted]
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	-	-

Section A7 Annex Point IIIA7.4.3.2	**Effects on reproduction and growth rate on an appropriate species of fish**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [...]**	
Detailed justification:	Dichlofluanid degrades rapidly in the aquatic environment. Therefore long term exposure of aquatic organisms to dichlofluanid from its application or service life as active substance in wood preservatives is improbable. Due to the use only up to hazard class 3 permanent contact of the treated wood to water is not intended, too.	
Undertaking of intended data submission [ ]	–	
	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	
Evaluation of applicant's justification **Conclusion**	[redacted]	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section 7.4.3.4 Annex Point IIIA XIII 2.4 — Effects on reproduction and growth rate with an invertebrate species

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] 1989, Influence of Dichlofluanid on the reproduction of *Daphnia magna* [redacted]	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
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. 202, Part II	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes, with regard to OECD guideline No. 202, Part II: test started with 20 animals divided into two groups of 10 animals each instead of at least 40, preferably divided into four groups of ten animals each; presence of eggs was not recorded	X
		3 METHOD	
3.1	**Test material**	As given in section 2 of dossier	
3.1.1	Lot/Batch number	Batch number: 1530 B	
3.1.2	Specification	As given in section 2 of dossier	
3.1.3	Purity	[redacted]	X
3.1.4	Composition of Product	-	
3.1.5	Further relevant properties	Water solubility: 1.3×10^{-3} g/l at 20 °C; Vapour pressure: 1.4×10^{-7} mbar at 20 °C; Stability in water: $t_{½} > 18$ h at 22 °C and pH 7	
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 means of GC.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	See table A7_4_3_4-1	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference	-	

Section 7.4.3.4 Annex Point IIIA XIII 2.4 — Effects on reproduction and growth rate with an invertebrate species

	substance		
3.4	**Testing procedure**		
3.4.1	Dilution water	See table A7_4_3_4-2	
3.4.2	Test organisms	See table A7_4_3_4-3	
3.4.3	Handling of offspring	Reproduction success was measured by controlling the number of young three times per week before renewal of test media. Dead offsprings were removed at the observation dates.	X
3.4.4	Test system	see table A7_4_3_4-4	X
3.4.5	Test conditions	see table A7_4_3_4-5	
3.4.6	Duration of the test	24 days	
3.4.7	Test parameter	Survival and reproduction rate of daphnids	X
3.4.8	Examination / Sampling	The mortality of adults and the number of young was controlled three times per week before renewal of test media. Dead animals and off-springs were removed at the observation dates. pH and oxygen concentration of test samples (control, solvent control, lowest (0.002 mg/l) and highest (1.0 mg/l) test concentration of test substance) were controlled at all treatment periods at the beginning and end of the respective periods.	X
3.4.9	Monitoring of TS concentration	Yes, Concentration of test substance in the test medium was determined for untreated (control with acetone), 0.002, 0.04 and 1.0 mg/l. Samples of 10 ml were taken on day 0, 3, 20, 22 of the test.	
3.4.10	Statistics	Statistical analysis of the reproduction rate was calculated for the individual daphnids. The Steel – Test (many – one rank test) was applied, since the data could not be assumed to follow a normal distribution.	
		4 RESULTS	
4.1	**Range finding test**	Not performed	
4.1.1	Concentrations	-	
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: 0.002, 0.008, 0.04, 0.2 and 1.0 mg/l	

Section 7.4.3.4 Annex Point IIIA XIII 2.4 — Effects on reproduction and growth rate with an invertebrate species

X

4.2.2 Actual concentrations of test substance

Measured concentrations of dichlofluanid (mg/l):

Nominal concentration (mg/l)	Day 0	Day 3	Day 20	Day 22
Control	< 0.005 / < 0.005	< 0.005 / < 0.005	< 0.005 / < 0.005	< 0.005 / < 0.005
0.04	< 0.005 / < 0.005	< 0.005 / < 0.005	< 0.005 / < 0.005	< 0.005 / < 0.005
1.0	0.4184 / 0.4432	< 0.005 / < 0.005	-	-

4.2.3 Effect data

The total numbers of living offspring per parent animal alive at test termination:

control	Solvent control	0.002 mg/l	0.008 mg/l	0.04 mg/l	0.2 mg/l	1.0 mg/l
58	87	79	64	65	45	-

At the highest test concentration (1.0 mg/l) no young daphnids were reproduced.

The number of dead parent animals (including time of death):

control	Solvent control	0.002 mg/l	0.008 mg/l	0.04 mg/l	0.2 mg/l	1.0 mg/l
2	**0**	**1**	**2**	**3**	**7**	**20**
1 day 10		1 day 3	1 day 10	1 day 6	4 day 3	10 day 3
1 day 22			1 day 22	1 day 15	1 day 13	2 day 6
				1 day 24	1 day 20	2 day 8
					1 day 24	4 day 10
						1 day 13
						1 day 15

Experiment was started with 10 daphnids per beaker (two replicates); on day 8 – 10, the number of daphnids was reduced to one daphnia per beaker (ten replicates, except at 1.0 mg/l, where only 6 daphnids remained alive on day 8).

No significant influence of the test substance on the reproduction rate was observed up to a concentration of 0.04 mg/l. At a concentration of 0.2 mg/l, a significant inhibition of the reproduction was observed.

The NOEC, taking into account survival and reproduction rate, was 0.04 mg/l. The LOEC on daphnia reproduction was 0.2 mg/l.

4.2.4 Concentration / response curve

No graph is given in the report

4.2.5 Other effects

-

Section 7.4.3.4 Annex Point IIIA XIII 2.4 — Effects on reproduction and growth rate with an invertebrate species

4.3	**Results of controls**	Mortality of control samples containing 0.01% acetone and samples without acetone proved to be low amounting to 0 and 20% for acetone–treated and untreated samples, respectively.	
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 effects of dichlofluanid on reproduction and growth rate to *Daphnia magna* were shown in a semi – static test which prolonged to 24 days. Test organisms were exposed to aqueous test medium containing the test substance at various concentrations. The mortality, the time of the first production of young, the number of young born and the signs of intoxication observed were compared with corresponding parameters in the controls. The study follows OECD guideline No. 202, Part II and shows no relevant deviations, but: Test started with 20 animals divided into two groups of 10 animals each instead of at least 40, preferably divided into four groups of ten animals each; Presence of eggs was not recorded	
5.2	**Results and discussion**	No significant influence of the test substance on the reproduction rate was observed up to a concentration of 0.04 mg/l. At a concentration of 0.2 mg/l, a significant inhibition of the reproduction was observed. Mortality of control samples containing 0.01% acetone and samples without acetone proved to be low amounting to 0 and 20% for acetone–treated and untreated samples, respectively. The few losses of daphnids were primarily due to mechanical damage of the daphnids during the water renewal procedures. The determination of the test substance in the test medium showed low analytical results. The rapid hydrolysis of the test substance at a pH > 7 was considered to be the cause of the differences between the nominal and measured concentrations.	X
5.2.1	NOEC	0.04 mg/l	
5.2.2	LOEC	0.2 mg/l	
5.2.3	EC_{50} (EC_x)	-	
5.3	**Conclusion**	Validity criteria can be considered as fulfilled. A dose response relationship can be seen from the experiment. At a concentration of 0.2 mg/l, a significant inhibition of the reproduction was observed.	
5.3.1	Reliability	2	
5.3.2	Deficiencies	Yes test started with 20 animals divided into two groups of 10 animals each	

Section 7.4.3.4 Annex Point IIIA XIII 2.4 — Effects on reproduction and growth rate with an invertebrate species

instead of at least 40, preferably divided into four groups of ten animals each;
presence of eggs was not recorded

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE [redacted]
Date	13/12/04
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	
Reliability	
Acceptability	
Remarks	
Date	**COMMENTS FROM ...** *(specify)*
Materials and Methods	*Give date of comments submitted*
Results and discussion	*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*
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_3_4-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	Yes 100 mg of the test substance were dissolved in 10 ml acetone. 0.1 ml from this stock solution were made up to 1000 ml test medium (= final stock solution, 1µg test substance / ml)
Concentration of vehicle	Concentration in solvent control: 0.01%
Vehicle control performed	Yes observation for mortality and reproduction rate was performed
Other procedures	-

Table A7_4_3_4-2: Dilution water

Criteria	Details
Source	Reconstituted water (25 ml of the below given stock solution to bidistilled water made up to 1 litre): $CaCl_2 * 2H_2O$ = 11.76 g/l, $MgSO_4 * 7H_2O$ = 4.93 g/l, $NaHCO_3$ = 2.59 g/l, KCl = 0.23 g/l
Salinity	-
Hardness	-
pH	8.0 – 8.2 (initial pH)
Ca / Mg ratio	$CaCl_2 * 2H_2O$ = 11.76 g/l $MgSO_4 * 7H_2O$ = 4.93 g/l
Na / K ratio	$NaHCO_3$ = 2.59 g/l KCl = 0.23 g/l
Oxygen content	8.8 mg/l (initial value)
Conductance	-
TOC	-
Holding water different from dilution water	No data

Table A7_4_3_4-3: Test organisms

Criteria	Details
Strain / Clone	*Daphnia magna*
Source	[redacted]
Age	< 24 hours
Breeding method	Standardized conditions
Kind of food	Mixture of yeast and algae (*Scenedesmus subspicatus*)
Amount of food	Day 0 – 8: algae and yeast each 200000 cells/ml, Day 8 – 15: algae and yeast each 300000 cells/ml, Day 15 – 24: algae and yeast each 600000 cells/ml
Feeding frequency	see above
Pretreatment	-
Feeding of animals during test	Yes see above

Table A7_4_3_4-4: Test system

Criteria	Details
Test type	Semi - static
Renewal of test solution	The treated and untreated test medium was renewed at day 3, 6, 8, 10, 13, 15, 17, 20 and 22 of the exposure period, preferably every Monday, Wednesday and Friday. By that a total of 10 treatments was performed.
Volume of test vessels	200 ml, on day 8 – 10: 50 ml
Volume/animal	20 ml
Number of animals/vessel	10, on day 8 – 10 number of daphnids was reduced to one daphnia per beaker
Number of vessels/concentration	two replicates, on day 8 – 10: ten replicates
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_3_4-5: Test conditions

Criteria	Details
Test temperature	21.5 – 22.5 °C, throughout the whole study
Dissolved oxygen	Mean value: 8.8 – 9.9 mg/l
pH	Mean value: 8.2 – 8.4
Adjustment of pH	No
Aeration of dilution water	Yes Before use, the test medium (= reconstituted water) was aerated until oxygen saturation
Quality/Intensity of irradiation	About 500 – 2000 Lux
Photoperiod	12 – 16 hours light / day

Table A7_4_3_4-6: Validity criteria for invertebrate reproduction test according to OECD Guideline 211

Test was performed according to OECD guideline 202, Part II

*	Fulfilled	Not fulfilled
Mortality of parent animals < 20% at test termination		
Mean number of live offspring produced per parent animal surviving at test termination ≥ 60		

Criteria for poorly soluble test substances	X	

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**	[redacted] 1999, Influence of Dimethylaminosulfanilid (DMSA) on Development and Emergence of Larvae of *Chironomus riparius* in a Water-Sediment System [redacted]
1.2	**Data protection**	Yes
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
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	Batch number: 203741099
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.

Section A7.4.3.5.1 Effects on sediment dwelling organisms

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

Chironomus riparius

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

Section A7.4.3.5.1 Effects on sediment dwelling organisms

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

Chironomus riparius

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 [redacted]
Materials and Methods	[redacted]
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	
	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