

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

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			Official use only
1 REFERENCE			
1.1 Reference	██████████, 1989, Toxicity of Dichlofluanide techn. (VM 90) for Rainbow Trout (<i>Salmo gairdneri</i>) with prolonged exposure (21 days), ██████████ ██████████, Report No. FF - 246 (unpublished), 1989-04-19.		
	An amendment of the report is currently (March 2004) under preparation.		X
1.2 Data protection	Yes		
1.2.1 Data owner	Bayer Crop Science AG		
1.2.2 Companies with letter of access	Bayer Chemicals AG		
1.2.3 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA		
2 GUIDELINES AND QUALITY ASSURANCE			
2.1 Guideline study	Yes		
	OECD guideline No. 204		
2.2 GLP	Yes		
2.3 Deviations	No		
3 MATERIALS AND METHODS			
3.1 Test material	Dichlofluanid techn. (90% premixture = VM 90)		
3.1.1 Lot/Batch number	Lot number: ██████████		
3.1.2 Specification	██████████ dichlofluanid		
3.1.3 Purity	██████████		
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	-		

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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	<p>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).</p> <p>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.</p> <p>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.</p>
3.4.8	Monitoring of TS concentration	<p>Yes</p> <p>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.</p>
3.4.9	Statistics	<p>Statistical analysis of results for a 21 – day LC₅₀ value and its corresponding 95% confidence limit was calculated using the method of sliding means.</p> <p>Body weights and length of the surviving fish of the various test concentrations at the end of the experiment were compared with those of the controls using t – tests.</p>

4 RESULTS

4.1	Limit Test	Not performed
4.1.1	Concentration	-
4.1.2	Number/ percentage of animals showing adverse effects	-
4.1.3	Nature of adverse effects	-
4.2	Results test substance	
4.2.1	Initial concentrations of test substance	<p>Nominal concentrations: 2.97, 6.40, 13.78, 29.70 and 63.98 µg a.i./l,</p>

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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 90) for rainbow trout (<i>Salmo gairdneri</i>) 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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		<p>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 LC₅₀ 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 (LC₅₀ = 15.2 µg/l) although exposure was kept constant during this flow-through study. The toxicity pattern is typical for dichlofluanid.</p> <p>No mortality or behavioural changes occurred in the controls. In the solvent control group, one fish died from cannibalism during the experiment.</p> <p>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.</p>	
5.2.1	21d-LC ₅₀	18.65 µg a.i./l; recalculation based on measured concentrations: 12.3 µg a.i./l	X
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	<p>The validity criteria are summarised in table A7_4_3_1-10.</p> <p>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.</p> <p>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.</p> <p>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).</p>	
5.3.1	Other Conclusions	-	
5.3.2	Reliability	2	
5.3.3	Deficiencies	<p>Yes,</p> <p>no method mentioned used for the determination of the test substance in the test medium (only reference to RA-No.)</p>	

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Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	13/07/06
Materials and Methods	<p>1.1 Applicant has submitted the amendment, which is stored and referenced with the original study.</p> <p>Accept applicant's version noting the following minor deviation:</p> <p>3.1.6 No method referred to for the determination of analytical concentrations of the test substance.</p>
Results and discussion	<p>Accept applicant's version noting the following minor deviation:</p> <p>4.2.2 Table A7_4_3_1_8 shows the recalculation of results based on mean measured concentrations. The 2 lowest nominal concentrations; 6.4 and 14 were measured on days 0 and 21 in accordance with the guideline. The nominal concentration of 14 showed an increase in measured concentration from the start to the end of the study (8.6 - 10). The 2 higher nominal concentrations; 30 and 64 were measured at the start, then on day 4 for 30 µg/l and day 1 for 64 µg/l. The concentration of the 64 µg/l nominal concentration fell from 46 on day 0 to 24 on day 1.</p> <p>4.2.4 Refers to nominal concentrations for effects when concentrations were not maintained within 80% of nominals.</p>
Conclusion	<p>Accept applicant's version with the following exception:</p> <p>5.2 In the results and discussion the applicant notes that; 'toxicity did not increase significantly after day 1 (LC₅₀ = 15.2 µg /l) although exposure was kept constant during this flow-through study.' The UK CA considers because of the limited number of measured concentrations and the apparent decline in concentration during the study, that the constant exposure claimed by the applicant is not supported.</p> <p>5.2.1 The endpoint reported is within the same order of magnitude as that reported for the acute LC₅₀ of 16.0 µg a.i./l reported for rainbow trout.</p>
Reliability	<p>Reliability = 2</p> <p>The lack of measured concentrations is considered a minor deficiency as the study follows the guideline and the UK CA does not consider there is any benefit in repeating the study.</p>
Acceptability	Acceptable
Remarks	All endpoints and data presented in the summary and tables have been checked against the original summary and are correct.
	COMMENTS FROM ...
Date	<i>Give date of comments submitted</i>

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Materials and Methods	<i>Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state</i>
Results and discussion	<i>Discuss if deviating from view of rapporteur member state</i>
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>
Reliability	<i>Discuss if deviating from view of rapporteur member state</i>
Acceptability	<i>Discuss if deviating from view of rapporteur member state</i>
Remarks	

Table A7_4_3_1-1: 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 (<i>Salmo gairdneri</i>)
Source	Test fish were obtained from the [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 [$\mu\text{g a.i./l}$] 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 ($\mu\text{g/l}$)			
	6.4	14	30	64
Sampling time	Measured concentration of dichlofluanid ($\mu\text{g/l}$)			
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 ($\mu\text{g/l}$)			
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 \geq 80% of initial concentration during test		X
Criteria for poorly soluble test substances	X	