

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

		Official use only
<b>1 REFERENCE</b>		
<b>1.1 Reference</b>	A. Ritter, 1989, Influence of Dichlofluanid on the reproduction of <i>Daphnia magna</i> , RCC Umweltchemie AG, Itingen, Switzerland, RCC Project No. 232841 (unpublished), 1989-05-24	
<b>1.2 Data protection</b>	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	
<b>2 GUIDELINES AND QUALITY ASSURANCE</b>		
<b>2.1 Guideline study</b>	Yes OECD guideline No. 202, Part II	
<b>2.2 GLP</b>	Yes	
<b>2.3 Deviations</b>	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
<b>3 METHOD</b>		
<b>3.1 Test material</b>	As given in section 2 of dossier	
3.1.1 Lot/Batch number	██████████	
3.1.2 Specification	As given in section 2 of dossier	
3.1.3 Purity	██████	X
3.1.4 Composition of Product	-	
3.1.5 Further relevant properties	Water solubility: $1.3 \times 10^{-3}$ g/l at 20 °C; Vapour pressure: $1.4 \times 10^{-7}$ mbar at 20 °C; Stability in water: $t_{1/2} > 18$ 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.	
<b>3.2 Preparation of TS solution for poorly soluble or volatile test substances</b>	See table A7_4_3_4-1	
<b>3.3 Reference substance</b>	No	
3.3.1 Method of analysis for reference	-	

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	substance		
<b>3.4</b>	<b>Testing procedure</b>		
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 offsprings 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.	
		<b>4 RESULTS</b>	
<b>4.1</b>	<b>Range finding test</b>	Not performed	
4.1.1	Concentrations	-	
4.1.2	Number/ percentage of animals showing adverse effects	-	
4.1.3	Nature of adverse effects	-	
<b>4.2</b>	<b>Results test substance</b>		
4.2.1	Initial concentrations of test substance	Nominal concentrations: 0.002, 0.008, 0.04, 0.2 and 1.0 mg/l	

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4.2.2 Actual concentrations of test substance

Measured concentrations of dichlofluanid (mg/l):

X

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

-

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4.3	<b>Results of controls</b>	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	<b>Test with reference substance</b>	Not performed	
4.4.1	Concentrations	-	
4.4.2	Results	-	
<b>5      APPLICANT'S SUMMARY AND CONCLUSION</b>			
5.1	<b>Materials and methods</b>	<p>The effects of dichlofluanid on reproduction and growth rate to <i>Daphnia magna</i> 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.</p> <p>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</p>	
5.2	<b>Results and discussion</b>	<p>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.</p> <p>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.</p> <p>The determination of the test substance in the test medium showed low analytical results. The rapid hydrolysis of the test substance at a pH &gt; 7 was considered to be the cause of the differences between the nominal and measured concentrations.</p>	X
5.2.1	NOEC	0.04 mg/l	
5.2.2	LOEC	0.2 mg/l	
5.2.3	EC <sub>50</sub> (EC <sub>x</sub> )	-	
5.3	<b>Conclusion</b>	<p>Validity criteria can be considered as fulfilled.</p> <p>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.</p>	
5.3.1	Reliability	2	
5.3.2	Deficiencies	Yes	
		test started with 20 animals divided into two groups of 10 animals each	

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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

<b>EVALUATION BY RAPPORTEUR MEMBER STATE</b>	
<b>Date</b>	13/12/04
<b>Materials and Methods</b>	Accept applicant's version noting the following minor deviations: <b>3.1.3</b> The purity was only [REDACTED] <b>3.4.3</b> and <b>3.4.8</b> Applicant states controlling the number, when counting the number is probably meant. <b>3.4.4</b> As identified by the applicant in 2.3, the test used 20 animals divided into 2 groups of 10, not 40 divided into 4 groups of 10 as in the guidance. <b>3.4.7</b> As identified by the applicant in 2.3, the presence of eggs was not recorded.
<b>Results and discussion</b>	<b>4.2.2</b> Concentrations were measured on days 0, 3, 20 and 22 in the control, the 0.04 mg/l concentrations, and on day 0 and 3 in the 1 mg/l. The only sampling point at which the measurement was above the limit of detection was the day 0, 1 mg/l at which the measurement was 0.4 mg/l.  The results have been reported for nominal concentrations that were not maintained. The UK CA consider that concentrations have not been maintained as required in the guideline.
<b>Conclusion</b>	<b>5.2</b> The low analytical results are not acceptable for this study
<b>Reliability</b>	Reliability = 3
<b>Acceptability</b>	Not acceptable  Concentration not being maintained throughout the study is a major deviation in conjunction with insufficient analytical data to allow for any statistical analysis of the reported effects.
<b>Remarks</b>	
<b>Date</b>	<b>COMMENTS FROM ... (specify)</b>
<b>Materials and Methods</b>	<i>Give date of comments submitted</i>
<b>Results and discussion</b>	<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>
<b>Conclusion</b>	<i>Discuss if deviating from view of rapporteur member state</i>
<b>Reliability</b>	<i>Discuss if deviating from view of rapporteur member state</i>
<b>Acceptability</b>	<i>Discuss if deviating from view of rapporteur member state</i>
<b>Remarks</b>	



**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 <sub>2</sub> * 2H <sub>2</sub> O = 11.76 g/l, MgSO <sub>4</sub> * 7H <sub>2</sub> O = 4.93 g/l, NaHCO <sub>3</sub> = 2.59 g/l, KCl = 0.23 g/l
Salinity	-
Hardness	-
pH	8.0 – 8.2 (initial pH)
Ca / Mg ratio	CaCl <sub>2</sub> * 2H <sub>2</sub> O = 11.76 g/l MgSO <sub>4</sub> * 7H <sub>2</sub> O = 4.93 g/l
Na / K ratio	NaHCO <sub>3</sub> = 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	<i>Daphnia magna</i>
Source	Animals were bred at RCC Umweltchemie AG
Age	< 24 hours
Breeding method	Standardized conditions
Kind of food	Mixture of yeast and algae ( <i>Scenedesmus subspicatus</i> )
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	<b>X</b>	