

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

		Official use only
		1 REFERENCE
1.1 Reference	Kern, M. E., Nieden, D., and Lam C. V. (2006): Chronic Toxicity of Dichlofluanid technical to the <i>Daphnia magna</i> Under Flow-Through Conditions. Bayer CropScience unpublished Report-No: EBDFX003.	
1.2 Data protection	Yes	
1.2.1 Data owner	LANXESS Deutschland GmbH	
1.2.2 Companies with letter of access	-	
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 FIFRA 72-4 (b) OPPTS Guideline 850.1300	
2.2 GLP	Yes	
2.3 Deviations	No significant deviations. HCL acid was administered to the study source water to lower the pH from approximately 7.9 to 7.0 in order to increase test material stability in the system.	
		3 METHOD
3.1 Test material	Dichlofluanid Technical	
3.1.1 Lot/Batch number	Batch number: ██████████	
3.1.2 Specification	As given in section 2	
3.1.3 Purity	██████ of active substance	
3.1.4 Composition of Product	-	
3.1.5 Further relevant properties	Dichlofluanid hydrolysis in water ($t_{1/2} = 25.6$ hours at pH 7 and 20 °C), non-volatile, water solubility: 1.58 mg/l at 20 °C. See study summary A3.	
3.1.6 Method of analysis	The analysis was performed using a gas chromatograph equipped with an electron-capture detector (GC/ECD); LOQ = 0.26 ppb. The method was validated by spiking dilution water with Dichlofluanid technical at 0.26-, 1.02-, 40.8-, and 102-ppb concentrations. Nine spikes were prepared and analyzed during method validation. The average recovery from 9 spikes was 99 % with a relative standard deviation (RSD) of 6 %.	
3.2 Preparation of TS solution for poorly soluble or volatile	See Table A7_4_3_4-1 A 400 mg a.i./L stock solution was prepared by adding approximately 205 mg of Dichlofluanid technical (adjusted for purity) to a 0.5-L	

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	test substances	quantity of dimethylformamide. The content was then stirred for approximately 0.25 hours. No precipitates were observed. The stock solution was used to dose the test aquaria via a modified proportional diluter system (Mount and Brungs, 1967). Additional stock solutions were prepared once a week during the course of the study.	
3.3	Reference substance	None	
3.3.1	Method of analysis for reference 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	See below "examination and sampling" (Chapter 3.4.8)	
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	21 days	
3.4.7	Test parameter	Adult daphnid survival, the mean young/aldult/reproduction, and the adult daphnid mean length.	
3.4.8	Examination / Sampling	<p>Dissolved oxygen, pH, alkalinity, hardness and conductivity were measured in all levels on Day 0 and weekly thereafter. Temperature was measured daily.</p> <p>All parent daphnids were observed daily for immobilization and sublethal effects. Dead organisms were recorded and removed daily. On the day of first release, neonates were counted and removed. On subsequent days, the neonates were counted and removed from the test chambers on a Monday-Wednesday-Friday schedule. The neonates were noted as present, if appropriate, on Tuesday, Thursdays and weekends. The neonates were counted on a light table as they were siphoned out of the test chamber, the count was recorded and the neonates were discarded.</p> <p>The body length and dry weight of parent daphnids was measured at test termination.</p>	
3.4.9	Monitoring of TS concentration	Samples of test solutions, including controls, were taken to determine measured Dichlofluanid test concentrations on Days 0, 9, 10, 15 and 21.	
3.4.10	Statistics	<p>For each analyzed parameter the following statistical tests were conducted:</p> <ol style="list-style-type: none"> 1) chi-square test to test the normality of the data set; 2) Bartlett's test for homogeneity of variances. To determine if the treatment groups were significantly different from the control, the reproduction data, survival data and parent daphnid growth data (length and dry weight) were analyzed by a one-way analysis of variance (ANOVA) followed by the Dunnett's test and William's test, or a nonparametric analysis if the assumptions of normality and/or homogeneity of variance were not met. 	

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Statistical analyses were conducted using PC based computer programs (TOXSTAT) developed by West, Inc. and Gulley (1994) with conclusions of statistical significance based on a 95 percent confidence level ($p \leq 0.05$).

4 RESULTS

4.1	Range finding test	Not performed. The test concentrations were determined based on historical data	
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	1.02, 2.56, 6.40, 16.0 and 40.0 µg/L test solution	
4.2.2	Actual concentrations of test substance	See Table A7_4_3_4-6. Mean measured concentrations are in brackets: control (<0.26), solvent control (<0.26), 1.02 (1.40), 2.56 (2.65), 6.40 (7.93), 16.0 (15.2) and 40.0 (35.9) µg a.i./L. This represents a range 90 to 137 percent of the nominal concentration for all levels (average 93 % with a RSD of 7 %).	X
4.2.3	Effect data	See Table A7_4_3_4-7. The mean number of live offspring produced per parent control animal surviving at the end of the test was > 60. A significant effect was noted at the 7.93, 15.2 and 35.9 µg a.i./L test levels resulting in a NOEC and LOEC of 2.65 and 7.93 µg a.i./L, respectively. The mean number of young per adult reproduction day ranged from 2.78 to 5.37 for the controls and all test levels.	
4.2.4	Concentration / response curve	Not given in the report	
4.2.5	Other effects	From day 9 to 14 adult daphnia in the highest level (35.9 µg a.i./L) of replicates C and D were pale in color. Daphnid length and dry weight: a significant effect was noted at the 7.93, 15.2 and 35.9 µg a.i./L test levels resulting in a NOEC and LOEC of 2.65 and 7.93 µg a.i./L, respectively. See Table A7_4_3_4-7.	
4.3	Results of controls	Not relevant adverse effects (control and solvent control)	
4.4	Test with reference substance	Not performed	
4.4.1	Concentrations	-	

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4.4.2	Results	-	
5 APPLICANT'S SUMMARY AND CONCLUSION			
5.1	Materials and methods	A 21-day chronic test starting with first instars of <i>Daphnia magna</i> (< 24 h old) was performed under flow-through conditions according to current guidelines (FIFRA 72-4 (b) and OPPTS Guideline 850.1300). This study was designed to establish a no-observed-effect-concentration (NOEC), a lowest-effect-observed-concentration (LOEC) and a Maximum Allowable Toxicant Concentration (MATC), which equals the geometric mean of the NOEC and LOEC. The NOEC is the highest concentration that causes no statistically verifiable adverse effects in the test population. The LOEC is the lowest concentration that produces at least one statistically significant ($p \leq 0.05$) adverse effect. The parameters measured in this study were daphnid survival (immobilization), growth and reproduction.	
5.2	Results and discussion	The 21-day exposure to Dichlofluanid technical resulted in a NOEC of 2.65 µg a.i./L and a LOEC of 7.93 µg a.i./L based on reproduction, length and dry weight. The MATC (Maximum Allowable Toxicant Concentration, which equals the geometric mean of the NOEC and LOEC) is 4.58 µg a.i./L.	X
5.2.1	NOEC	2.65 µg/l	
5.2.2	LOEC	7.93 µg/l	
5.2.3	EC ₅₀ (EC _x)	Not calculated, it is above the highest concentration tested >40.0 (35.9, measured) µg a.i./L	
5.3	Conclusion	There is a dose response relationship in concentrations above 7.93 µg/l. Validity criteria can be considered fulfilled. See also Table A7_4_3_4-8.	X
5.3.1	Reliability	Reliability indicator 1	
5.3.2	Deficiencies	No	

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Evaluation by Competent Authorities									
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted								
	EVALUATION BY RAPPORTEUR MEMBER STATE								
Date	08/05/2006								
Materials and Methods	The Applicant's version is acceptable with the following comments: 3.4.4: Table A7_4_3_4-4 Test System, is incorrect for 3 criteria and should be replaced by <table border="1" style="margin-left: 20px;"> <thead> <tr> <th>Criteria</th> <th>Details</th> </tr> </thead> <tbody> <tr> <td>Volume/animal</td> <td>900 ml/10 or 90 ml/daphnid</td> </tr> <tr> <td>Number of animals/vessel</td> <td>10</td> </tr> <tr> <td>Number of vessels/concentration</td> <td>4</td> </tr> </tbody> </table>	Criteria	Details	Volume/animal	900 ml/10 or 90 ml/daphnid	Number of animals/vessel	10	Number of vessels/concentration	4
Criteria	Details								
Volume/animal	900 ml/10 or 90 ml/daphnid								
Number of animals/vessel	10								
Number of vessels/concentration	4								
Results and discussion	The Applicant's version is acceptable with the following comments: 4.2.2: The measured values reported were outside the acceptable $\pm 20\%$ range from the nominal with up to 137 % reported. However, the results presented were all based on the mean measured levels and are acceptable.								
Conclusion	The Applicant's version is acceptable with the following comments: 5.2: The results presented are based on the mean measured concentrations. 5.3: Table A7_4_3_4-8 incorrectly lists the validity criteria for 211 as including: 'Test substances maintained within $\pm 20\%$ of mean measured values (otherwise determination of effects levels based on mean measured concentrations)' and that this has been fulfilled. The OECD 211 guideline states that the test substance should be maintained within $\pm 20\%$ of the nominal. This study was not carried out to OECD guideline, but the results (based on flow-through) are based on mean measured values, which is considered by the UK CA to be appropriate for this endpoint.								
Reliability	1								
Acceptability	Acceptable								
Remarks	No reduction in reliability is considered necessary based on the use of mean measured concentrations because this is a difficult substance to maintain and the data has been adequately recorded.								
Date	COMMENTS FROM ... (specify)								
Materials and Methods	<i>Give date of comments submitted</i>								
Results and discussion	<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>								
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>								

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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	<i>Discuss if deviating from view of rapporteur member state</i>

Table A7_4_3_4-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	Yes, dimethylformamide
Concentration of vehicle	205 mg of Dichlofluanid technical (adjusted for purity) to a 0.5-L quantity of dimethylformamide
Vehicle control performed	Yes
Other procedures	-

Table A7_4_3_4-2: Dilution water

Criteria	Details
Source	Spring water blended with reverse osmosis water to produce hard (target = 160 to 180 mg/L) water.
Salinity	Not stated
Alkalinity	100 to 160 mg/L as CaCO ₃ (measured during the test)
Hardness	166 to 172 mg/L as CaCO ₃ (measured during the test)
pH	7.2 to 8.1 (measured during the test)
Ca / Mg ratio	Not stated
Na / K ratio	Not stated
Oxygen content	7.0 to 8.8 mg/L (measured during the test)
Conductance	402 to 472 µmhos/cm (measured during the test)
TOC	Not stated
Holding water different from dilution water	No

Table A7_4_3_4-3: Test organisms

Criteria	Details
Strain / Clone	<i>Daphnia magna</i>
Source	In house <i>daphnid</i> culture
Age	First-instar daphnids < 24 hours old
Breeding method	<p>The water in the culture vessels was renewed three times per week. <i>Daphnia</i> culture techniques were based on those described by ASTM (1997). The culture area was maintained on a 16-hour daylight photoperiod, light intensity of approximately 40-70 foot-candle, and a temperature of $20 \pm 2.0^{\circ}\text{C}$. The water used to culture the <i>Daphnia</i> is from the same source as the water used in the test (had blended water, referred to as dilution water).</p> <p>The <i>Daphnia</i> subculture for the test was initiated on October 13, 2005. The <i>Daphnia</i> in the subculture were held under the same conditions as the culture.</p>
Kind of food	The daphnids were fed a combination of green algae (<i>Pseudokirchneriella subcapitata</i>) and blended Tetrafin® flaked fish food.
Amount of food	Not stated
Feeding frequency	Daphnids were fed algae (<i>Pseudokirchneriella subcapitata</i>) three times per day during the week and twice-three times daily on the weekends. <i>Daphnia</i> were also fed a slurry of Tetrafin® on Monday, Wednesday and Friday. The algae was dispensed equally to all vessels at a minimum rate of 2×10^8 algal cells/L of test solution. The blended Tetrafin® was dispensed equally to all vessels at 0.02 mg/L or less.
Pretreatment	No
Feeding of animals during test	Yes. See above

Table A7_4_3_4-4: Test system

Criteria	Details
Test type	Flow-through
Renewal of test solution	The test vessel turnover rate was approximately 22 turnovers per day
Volume of test vessels	300 ml
Volume/animal	ten 400-ml beakers for a total of 20 neonates
Number of animals/vessel	20 in-star daphnids
Number of vessels/ concentration	4
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_3_4-5: Test conditions

Criteria	Details
Test temperature	20 °C (range = 19.6 to 21.1°C)
Dissolved oxygen	7.0 to 8.8 mg/L, representing 77 to 97 percent saturation at 20°C
pH	7.2 to 8.1
Adjustment of pH	Yes; due to the limited solubility and stability of this test material in the dilution water, an adjustment to the pH of the test water was made as it entered the diluter system via the addition of HCl. This injection of HCl had no biological impacts on the test organisms.
Aeration of dilution water	No
Quality/Intensity of irradiation	The lighting was 443-699 lux (mean 611 lux)
Photoperiod	16-hour daylight and a 8- hour darkness

Table A7_4_3_4-6: Measured test concentrations of Dichlofluanid technical during the 21-day exposure of the *Daphnia magna*

Nominal Concentrations (µg a.i./L)	Mean Measured Concentrations (µg a.i./L)						Standard Deviation	Percent of Nominal
	Day 0	Day 9	Day 10	Day 15	Day 21	Mean		
Control	<0.26	0.85	<0.26	<0.26	<0.26	NA	NA	NA
Solvent Control	<0.26	0.43	<0.26	<0.26	<0.26	NA	NA	NA
1.02	1.40	1.34	NA	1.44	1.42	1.40	0.04	137
2.56	2.35	2.79	NA	2.82	2.64	2.65	0.21	104
6.40	9.69	6.83	NA	7.39	7.82	7.93	1.24	124
16.0	11.7	16.1	NA	17.0	16.1	15.2	2.37	95
40.0	28.9	34.4	NA	40.6	39.6	35.9	5.39	90
Limit of Quantitation = 0.26 ug/L				NA = samples purposely not taken and measured				

Table A7_4_3_4-7: Results of reproduction test

Endpoint results	Immobilization	Time to first brood	Neonates/ adult reproduction day	Adult body length	Adult dry weight	Sublethal effects
Highest Concentration Without an Effect (NOEC)	≥ 35.9	≥ 35.9	2.65	2.65	2.65	15.2
Lowest Concentration With an Effect (LOEC)	> 35.9	> 35.9	7.93	7.93	7.93	35.9
Maximum Acceptable Toxicant Concentration (Geometric mean of NOEC and LOEC)	≥ 35.9	≥ 35.9	4.58	4.58	4.58	23.4

Table A7_4_3_4-8: Validity criteria for invertebrate reproduction test according to OECD Guideline 211

	Fulfilled	Not fulfilled
Mortality of parent animals < 20% at test termination	X	
Mean number of live offspring produced per parent animal surviving at test termination ≥ 60	X	
Test substance concentrations maintained within ± 20% of mean measured values (otherwise determination of effect levels based on mean measured concentrations)	X	
Criteria for poorly soluble test substances	X	