Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA VII.7.2 *Daphnia magna*

			Official use only
		1 REFERENCE	
1.1	**Reference**	A.D., Forbis, 1986, Acute flow-through toxicity of Preventol A4-S to *Daphnia magna*, ABC (Analytical Bio-Chemistry Laboratories) INC., Columbia, Missouri, USA, Report No. 778 (unpublished), 1986-08-26	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer Chemicals AG	
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, U.S.-EPA, Ecological Research Series EPA-660/3-75-009, (April 1975)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No, the study is comparable to OECD guideline No. 202	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2 of dossier	
3.1.1	Lot/Batch number	Lot number: [redacted]	
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	HPLC	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	See table A7_4_1_2-1	
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_1_2-2	

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3.4.2	Test organisms	see table A7_4_1_2-3	X
3.4.3	Test system	see table A7_4_1_2-4	
3.4.4	Test conditions	see table A7_4_1_2-5	
3.4.5	Duration of the test	48 hours	
3.4.6	Test parameter	Mortality and behavioural observation	
3.4.7	Sampling	Mortality and behavioural observation was performed at 24 and 48 hours; pH and dissolved oxygen concentration of test samples (control, low, middle and high concentrations of test substance) were controlled at 0 and 48 hours	X
3.4.8	Monitoring of TS concentration	Yes, at 0 and 48 hours	X
3.4.9	Statistics	Statistical analysis was obtained by employing a computerized program. The LC_{50} values were calculated using the moving average method.	
		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: 0.12, 0.19, 0.40, 0.69, 1.6 mg/l	
4.2.2	Actual concentrations of test substance	Measured concentrations (mean values): 0.071, 0.099, 0.24, 0.35, 1.0 mg/l	X
4.2.3	Effect data (Immobilisation)	see table A7_4_1_2-6 and table A7_4_1_2-7	X
4.2.4	Concentration / response curve	No graph is given in the report	X
4.2.5	Other effects	Abnormal/behavioural responses (e.g. surfacing, quiescence and bottom orientation) were noted among the daphnids in the 0.099, 0.24, 0.35 and 1.0 mg/l test substance concentrations.	
4.3	**Results of controls**	No mortality occurred in the controls	
4.4	**Test with**	Not performed	

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	reference substance	
4.4.1	Concentrations	-
4.4.2	Results	-

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Acute toxicity test to *Daphnia magna* was performed in accordance with guideline U.S.-EPA, Ecological Research Series EPA-660/3-75-009, (April 1975). The test, performed in a flow-through system, prolonged to 48 hours. Comparison with OECD guideline No. 202 shows no relevant deviations.
5.2	**Results and discussion**	A LC_{50} value of 0.57 mg/l at 24 hours was shown in the test. The result is based on measured test concentrations used for statistical analysis. The 48-hour no-effect concentration was 0.071 mg/l, based on the lack of mortality and abnormal effects. No mortality occurred in the controls. The determination of the test substance concentrations in the test system showed low analytical results.
5.2.1	LC_0	0.099 mg/l after 24 h and 0.071 after 48 h
5.2.2	LC_{50}	0.57mg/l after 24 h, and 0.42 mg/l after 48 h
5.2.3	LC_{100}	> 1.0 mg/l after 24 h, and 1.0 mg/l after 48 h
5.3	**Conclusion**	The validity criteria are summarised in table A7_4_1_2-8. The measured concentrations of test substance are not ≥ 80% of nominal concentrations during the test. The differences between the nominal and measured concentrations were likely due to the fact that dichlofluanid is very rapidly hydrolysed in aqueous solutions. A concentration/response curve is not available but a dose – response relationship can be seen from the experiment.
5.3.1	Reliability	2
5.3.2	Deficiencies	Yes It must be noted that the LC_{50} value was calculated instead of the EC_{50} value. Therefore the EC_{50} value based on immobilisation is lower than 0.42 mg/l after 48 hours. Information is incomplete about test organism, No concentration/response curve available

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Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	28/01/05
Materials and Methods	Accept applicant's version noting the following deviations: **3.1.3** The purity was only [redacted]. **3.4.2** Information about the test organism is incomplete, there is no description of the pre-treatment, although this is highlighted by the applicant in 5.3.2. **3.4.7** Sample storage times before analysis were not given. **3.4.8** Concentrations were only measured at 0 and 48 h.
Results and discussion	Accept applicant's version noting the following deviations: **4.2.2** Measured concentrations increased during the study but were significantly lower than initial nominals as identified by the applicant in 5.3. Averaged measured concentrations were 64 ± 9 % and 50 ± 4 % of nominals, for the 0 and 48 h samples, respectively. The study author suggests the difference between nominal and measured concentrations was likely to be due to hydrolysis of Preventol A4-S in the test water. **4.2.3** LC_{50}s were given not EC_{50}s for immobilisation as in OECD 202, although this is highlighted by the applicant in 5.3.2. Mortality is not defined in the study. **4.2.4** Slope of the concentration/response curve with 95 % confidence limits was not provided, due to no graph being in the study. A graph could have been supplied from the data.
Conclusion	Accept applicant's version
Reliability	Reliability = 2
Acceptability	Acceptable The lack of sampling for measured concentrations is considered a minor deficiency as it is inline with the guideline. The apparent increase in dichlofluanid during the test is also considered to be a minor deficiency.
Remarks	All endpoints and data presented in the summary and tables have been checked against the original summary and are correct.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*

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

Table A7_4_1_2-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes special device (mixing box)
Vehicle	Yes dimethylformamide was used in the preparation of all working stock solutions
Concentration of vehicle	Volume for preparation of stock solution: 100 ml
Vehicle control performed	Yes mortality and behavioural observation was performed in solvent control
Other procedures	-

Table A7_4_1_2-2: Dilution water

Criteria	Details
Source	Aerated, aged ABC well water
Alkalinity ($CaCO_3$)	325-375 mg/l
Hardness ($CaCO_3$)	225-275 mg/l
pH	7.8 – 8.3
Ca / Mg ratio	-
Na / K ratio	-
Oxygen content	9.2- 10.1 mg/l
Conductance	700 µmhos/cm
Holding water different from dilution water	No

Table A7_4_1_2-3: Test organisms

Criteria	Details
Strain	*Daphnia magna*
Source	ABC Laboratories in-house culture
Age (at start of the study)	< 24 – hours old
Breeding method	-
Kind of food	Suspension of algae (*Selenastrum capricornutum*) supplemented with a yeast suspension
Amount of food	-
Feeding frequency	-
Pretreatment	-
Feeding of animals during test	During the holding period daphnids were fed with the above named kind of food

Table A7_4_1_2-4: Test system

Criteria	Details
Renewal of test solution	Flow-through system: aerated, aged ABC well water was delivered to each test chamber at a rate of 125 ml/chamber /10 minutes, an amount which was sufficient to replace the 1-liter test volume approximately 19 times in a 24-hour period.
Volume of test vessels	1 l
Volume/animal	100 ml
Number of animals/vessel	10
Number of vessels/ concentration	4 (4 replicate test chambers, i.e. 40 daphnids were used per concentrations)
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_2-5: Test conditions

Criteria	Details
Test temperature	20 – 21 °C
Dissolved oxygen	8.3 – 8.7 mg/l
pH	8.2 – 8.3
Adjustment of pH	No
Aeration of dilution water	Yes pretreatment
Quality/Intensity of irradiation	50 – 70 footcandles
Photoperiod	16 – hour daylight photoperiod, with 30 minutes dawn and dusk transition periods

Table A7_4_1_2-6: Mortality data

Test Substance Concentration (effective) [1] [mg/l]	Mortality of *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	8.3	8.2	20
Solvent control	0	0	0	0			
0.071	0	0	0	0	8.5	8.3	20
0.099	0	1	0	2.5			
0.24	1	2	2.5	5	8.5	8.3	20
0.35	3	16	7.5	40			
1.0	38	40	95	100	8.7	8.3	20

[1] Test substance concentrations are mean measured concentrations

Table A7_4_1_2-7: Effect data *

	LC_{50} [1]	95 % c l.	LC_0 [1]	LC_{100} [1]
24 h [mg/l]	0.57	0.51 – 0.67	0.099	> 1.0
48 h [mg/l]	0.42	0.37 – 0.47	0.071	1.0

[1] Effect data are based on measured concentrations

* The LC_{50} value was calculated instead of the EC_{50} value

Table A7_4_1_2-8: 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	X	