Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA VII.7.1 *Lepomis macrochirus*

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted], 1986, Acute Flow – Through Toxicity of Preventol A4-S to Bluegill Sunfish (*Lepomis macrochirus*), [redacted], Report No. [redacted] (unpublished), 1986-08-25	
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, (1975)	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes, after comparison with OECD guideline No. 203: Observation for mortality was not made in blank control	
		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_1-1	
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_1-2	

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3.4.2	Test organisms	see table A7_4_1_1-3	X
3.4.3	Test system	see table A7_4_1_1-4	
3.4.4	Test conditions	see table A7_4_1_1-5	
3.4.5	Duration of the test	96 hours	
3.4.6	Test parameter	Mortality and sublethal responses	
3.4.7	Sampling	Observations for mortality and sublethal responses were made once every 24 hours (each test level and acetone solvent control). Dead individuals were removed at each observation period. Temperature, dissolved oxygen and pH were measured in the solvent control, the low and the highest test concentration which contained surviving fish at 0, 48 and 96 hours.	X
3.4.8	Monitoring of TS concentration	Yes, at 0 and 96 hours	X
3.4.9	Statistics	Statistical analysis of results for 24, 48, 72 and 96 – hour LC_{50} values and their corresponding 95% confidence limits was obtained by employing a LC_{50} computerized program using the binomial, the moving average and the probit 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: 1.0, 0.5, 0.25, 0.125 and 0.06 mg/l	
4.2.2	Actual concentrations of test substance	Measured concentrations (mean values): 0.50, 0.25, 0.10, 0.05 and 0.024 mg/l	X
4.2.3	Effect data (Mortality)	see table A7_4_1_1-6 and table A7_4_1_1-7	
4.2.4	Concentration / response curve	No graph is given in the report	
4.2.5	Other effects	Sublethal/behavioural responses (e.g. loss of equilibrium, bottom orientation and rapid respiration) were observed in the 0.10 and 0.05	

		mg/l test levels.	
4.3	**Results of controls**		X
4.3.1	Number/ percentage of animals showing adverse effects	No mortality occurred in the solvent control	
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**	A 96 - hour flow - through study was conducted in accordance with the guideline U.S.-EPA, Ecological Research Series EPA-660/3-75-009, (1975) in order to estimate the acute toxicity of dichlofluanid to bluegill sunfish (*Lepomis macrochirus*). Comparison with OECD guideline No. 203 shows no relevant deviations except that observation for mortality was not made in blank control.	
5.2	**Results and discussion**	A 96 – hour LC_{50} value was calculated to be 0.030 mg/l with 95% confidence limits ranging from 0.024 to 0.050 mg/l. The result is based on the measured test concentrations of dichlofluanid. A 96 – hour no effect concentration of dichlofluanid was determined to be < 0.024 mg/l, because all test concentrations elicited total or partial mortality. No mortality occurred in the solvent control. The determination of the test substance concentrations in the test system showed low analytical results.	
5.2.1	96h-LC_0	< 0.024 mg/l	X
5.2.2	96h-LC_{50}	0.030 mg/l	
5.2.3	96h-LC_{100}	0.05 mg/l	
5.3	**Conclusion**	The validity criteria are summarised in table A7_4_1_1-8. 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.	

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5.3.1	Other Conclusions	-
5.3.2	Reliability	2
5.3.3	Deficiencies	Yes, observation for mortality was not made in blank control, no graph is given in the report

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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** Fish were longer than recommended in OECD 203 (4.6 cm as opposed to 2 ± 1 cm). For the pre-treatment fish were without food for 72 h before the test instead of 24 h. **3.4.7** Sample storage times before analysis was not given. **3.4.8** Concentrations were only measured at 0 and 96 h.
Results and discussion	Accept applicant's version noting the following deviations: **4.2.2** Concentrations were only measured at 0 and 96 h. OECD 203 requires evidence that the concentration has been satisfactorily maintained, the UK CA does not consider that this has been demonstrated. Averaged measured concentrations increased during the study and were significantly lower than initial nominals as identified by the applicant in 5.3. Measured concentrations were 36 ± 5 % and 49 ± 5 % of nominals, for the 0 and 96 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. Some poor recoveries are indicated in Table 3 in the study. **4.3** Mortality was not observed for in blank control, this has been highlighted by the applicant in 5.3.3.
Conclusion	Accept applicant's version with the comment that: **5.2.1** The LC_0 was not established as there were effects below the lowest concentration.
Reliability	Reliability = 2
Acceptability	Acceptable The lack of sampling for measured concentrations is considered a deficiency as there is not considered to be enough evidence to show that concentrations were maintained. The apparent increase in dichlofluanid during the test is considered a deficiency. The inability of the study to produce a dose-response curve is also considered as a deficiency. These deficiences are not considered to be sufficiently major to reduce the reliability indicator below 2 and justify a repeat of the study.
Remarks	All endpoints and data presented in the summary and tables have been checked against the original summary and are correct.
	COMMENTS FROM ...

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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_1-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	Yes A diluter stock solution (17.500 mg/l) was prepared by dissolving 1.750 g of dichlofluanid in 100 ml of acetone.
Concentration of vehicle	Concentration in solvent control: 0.05 ml/l
Vehicle control performed	Yes Observation for mortality and sublethal responses was performed in solvent control
Other procedures	-

Table A7_4_1_1-2: Dilution water

Criteria	Details
Source	ABC well water
Alkalinity	325 – 375 mg/l
Hardness	225 – 275 mg/l
pH	7.8 – 8.3
Oxygen content	9.2 – 10.1 mg/l (after aeration)
Conductance	700 µmhos/cm
Holding water different from dilution water	No

Table A7_4_1_1-3: Test organisms

Criteria	Details
Species/strain	Bluegill Sunfish (*Lepomis macrochirus*)
Source	Test fish were obtained from Osage Catfisheries, Missouri and maintained at ABC Laboratories in ABC well water.
Wild caught	No
Age/size	Bluegill sunfish used as control group: mean weight of 1.5 (± 0.4) g and a mean standard length of 46 (± 4.5) mm.
Kind of food	The fish were maintained at ABC Laboratories and were fed newly hatched brine shrimp or a commercially available trout food
Amount of food	-
Feeding frequency	Daily
Pretreatment	72 hours before initiation of test, fish were placed in the temperature acclimation unit and held without food during this time.
Feeding of animals during test	No

Table A7_4_1_1-4: Test system

Criteria	Details
Test type	Flow-through
Renewal of test solution	1 litre of test solution or control water was delivered to the test vessels at an average rate of 15 times per hour over the course of the study. This flow rate was sufficient to replace the 15 litre volume within the test chambers 24 times per day.
Volume of test vessels	15 l
Volume/animal	750 ml
Number of animals/vessel	20
Number of vessels/ concentration	1
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_1-5: Test conditions

Criteria	Details
Test temperature	22 – 23 °C
Dissolved oxygen	8.8 – 9.1 mg/l
pH	7.9 – 8.2
Adjustment of pH	No
Aeration of dilution water	Yes (pretreatment)
Intensity of irradiation	-
Photoperiod	Laboratory environment was maintained on a 16-hour daylight photoperiod

Table A7_4_1_1-6: Mortality data

Test Substance Measured Concentration [mg/l][1]	**Mortality**							
	Number				**Percentage**			
	24 h	**48 h**	**72 h**	**96 h**	**24 h**	**48 h**	**72 h**	**96 h**
Solvent control	0	0	0	0	0	0	0	0
0.024	4	4	4	4	20	20	20	20
0.05	12	19	19	20	60	95	95	100
0.10	19	20	20	20	95	100	100	100
0.25	20	20	20	20	100	100	100	100
0.50	20	20	20	20	100	100	100	100
Temperature [°C]	22 - 23							
pH	7.9 – 8.2							
Oxygen [mg/l]	8.8 – 9.1							

[1] Test substance concentrations are mean measured concentrations

Table A7_4_1_1-7: Effect data

	48 h [mg/l][1]	**95 % c l.**	**96 h [mg/l][1]**	**95 % c.l.**
LC_0	< 0.024	-	< 0.024	-
LC_{50}	0.031	0.026 – 0.037	0.030	0.024 – 0.05
LC_{100}	0.10	-	0.05	-

[1] Effect data are based on measured concentrations

Table A7_4_1_1-8: Validity criteria for acute fish test according to OECD Guideline 203

	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	