Section A7.2.3.2 Aged residues soil leaching study

Annex Point IIIA XII 1.3

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	02 04 04
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	
	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*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_2_3_2-1: Classification and physico-chemical properties of soils used

	Soil 1	Soil 2	Soil 3	Soil 4	Soil 5
Soil order					
Soil series					
Classification	Sand	Sandy loam	Silt loam	Silty Clay loam	Mixed sand
Location	Vero Beach, Florida	Stanley, Kansas	Stanley, Kansas	Hagerstown Maryland	Vero Beach, Florida
Horizon					
Sand [%]	92	54	13	4	98
Silt [%]	6	37	63	53	0
Clay [%]	2	9	24	43	2
Organic matter	4.4	1.8	2.7	2.1	0.8
Carbonate as $CaCO_3$					
insoluble carbonates [%]					
pH (in 0.01 M $CaCl_2$)	6.5	4.5	6.4	6.7	7.3
Cation exchange capacity (MEQ/100 g)	Not available	16	21.2	28.6	14
Extractable cations (MEQ/100 g)					
Ca					
Mg					
Na					
K					
H					
Special chemical/mineralogical features					
Clay fraction mineralogy					

Table A7_2_3_2-2: Distribution of aged tebuconazole residues following soil column leaching (Figures are in % of radioactivity applied to column)

	Soil 1	Soil 2	Soil 3	Soil 4	Soil 5
30 day aged samples					
Eluate	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Aged layer	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
0-6 cm	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
6-12 cm	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
12-18 cm	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
18-24 cm	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
24 + cm	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
^{14}C recovered	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
90 day aged samples					
Eluate	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Aged layer	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
0-6 cm	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
6-12 cm	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
12-18 cm	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
18-24 cm	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
24+ cm	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
^{14}C recovered	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

Section A7.3.1 Phototransformation in Air (Calculation method)

Annex Point IIIA VII5

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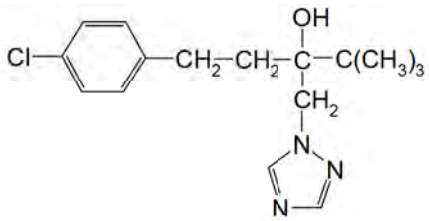
1 REFERENCE

1.1	**Reference**	E. Hellpointner. Calculation of the chemical lifetime of tebuconazole in the troposphere. Bayer AG , Crop Protection Research, Environmental Research, Institute for Metabolism Research, PF-Report No. 3808, Monheim, January 12, 1993
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Up to now no guideline available
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

3.1	**Test material**	Tebuconazole = (+-) alpha[2-(4-chlorophenyl)ethyl].alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol
3.2	**Structural formula**	
3.3	**Calculation program**	The calculation was performed using the AOP (Atmospheric Oxidation Program) Version 1.40 (August 1991). The program is an adoption of the estimation methodology from Atkinson* developed by Syracus Research Corporation, Chemical Hazard Assessment Division, Merrill Lane, Syracuse, NY 13210 *References: Int. J. Kinet. **19**: 799-828 (1987) & Env. Toxic Chem. **7**: 435-442 (1988).

Section A7.3.1 Phototransformation in Air (Calculation method)

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3.4	**Regarded mechanisms**	1) hydrogen abstraction by OH radicals from aliphatic bonds, 2) addition of OH radicals to aromatic rings (assumed values) 3) reaction of OH with OH bonds The 1, 2, 4-Triazole ring was simulated by a methyl group, because no adequate structural element was available.
3.5	**Other mechanisms**	The program also is able to calculate the ozone reactivity. This is only possible for olefins and acetylene's`. OH reactivity will strongly predominate the degradation in air for most other chemical structures (including that of tebuconazole).
3.6	**Mean daily OH concentration in air**	5 10^5 OH radicals per cm^3.
3.7	**Transformation products**	Not covered by the calculation method. Nevertheless oxidative attacks of the OH radicals to the molecule are assumed. End product of this oxidative degradation in the air will be H_2O, CO_2 and Nitrogen oxides.
		4 RESULTS
4.1	**Overall OH-reactivity rate constant**	6.0618 10^{12} cm^3 molecule $^{-1}$ s^{-1}
4.2	**Overall tropo-spheric half–life**	2.6 days
4.3	**Specification of the transformation products**	Not in the scope of this model calculation
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The tropospheric half-live of tebuconazole was estimated using die AOP program from W. Meylan & E. Howard from the Syracuse Institute. The program is based on a well established QSR developed by Atkinson. Because for 1, 2, 4–triazole no elements for calculation were available, conservative estimations were done for the triazole group and the related factors for the OH addition to the substituted aromatic benzene ring.
5.2	**Results and discussion**	Using the under 5.1 mentioned estimations, an overall tropospheric half–life for 2.6 days was estimated. The Atkinson calculation method sums up the reactivity towards OH radicals of all structural elements. Therefore leaving out the aromatic elements and the methyl group which was used to simulate the 1, 2, 4-triazole ring the overall rate constant still would be 4.156 10^{12} cm^3 molecule $^{-1}$ s^{-1}. This value results in a tropospheric half-live of 3.8 days which can be regarded as the worst case. As a consequence the tropospheric half-life of tebuconazole can be defined as: < 4 days.
5.2.1	k_{OH}	6.0618 10^{12} cm^3 molecule $^{-1}$ s^{-1}
5.2.2	$t_{1/2air}$	2.6 days

Section A7.3.1 Phototransformation in Air (Calculation method)

Annex Point IIIA VII5

5.3	**Conclusion**	Concerning the overall relevance of the atmospheric fate of tebuconazole the very low vapour pressure of the compound has to be taken into account. Air will not be an environmental compartment of concern for tebuconazole used in wood preservatives. Based on the model calculation nevertheless small amounts that maybe volatise can be regarded as well degradable by oxidation via OH-radicals (Half-life.< 4 days)
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	[redacted]

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*02 04 04*
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

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

Section 7.3.2 Annex Point IIIA 12.3	Fate and behaviour in air, further studies	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	There were no further studies submitted on the fate and behaviour in air. Due to the very low vapour pressure of tebuconazole, air is not a relevant compartment for the active.	
Undertaking of intended data submission [ ]	–	
	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. September 2004*	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1

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		1 REFERENCE
1.1	**Reference**	[redacted], 1987. Acute Toxicity of HWG 1608 (technical grade) to rainbow trout (*Salmo gairdneri*) und flow through conditions. [redacted] Toxicology Report No. 954, [redacted] No. #BW-87-5-2394, September 14. 1987
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes / EPA Reference No. 72-1, also reference to: "Protocol for Freshwater Fish Flow-Through Acute Toxicity Test Under FIFRA Guidelines (November 1986), closely following the "Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians (ASTM, 1980)
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]
		3 MATERIALS AND METHODS
3.1	**Test material**	HWG 1608 (tebuconazole) technical grade
3.1.1	Lot/Batch number	Lot number #[redacted]
3.1.2	Specification	Two test substances used with different purity
3.1.3	Purity	[redacted] and [redacted] of active substance
3.1.4	Composition of Product	-
3.1.5	Further relevant properties	-
3.1.6	Method of analysis	HPLC reversed phase
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Tebuconazole was dissolved in acetone to 23.2 mg a.i./ml. A peristaltic pump was used to deliver this stock solution to a diluter system where it was appropriately diluted to provide the desired exposure concentration range.
3.3	**Reference substance**	No
3.3.1	Method of analysis	-

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1

	for reference substance	
3.4	**Testing procedure**	
3.4.1	Dilution water	See table A7_4_1_1-2
3.4.2	Test organisms	Rainbow trout, see table A7_4_1_1-3
3.4.3	Test system	Flow through 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
3.4.7	Sampling	24, 48, 72 and 96 hours, replicates and sample storage before analysis
3.4.8	Monitoring of TS concentration	Before the test and at each exposure time, samples were taken.
3.4.9	Statistics	Prohibit analysis to calculate the LC 50
		4 RESULTS
4.1	**Limit Test**	yes
4.1.1	Concentration	10 mg, 2.7 mg/l
4.1.2	Number/ percentage of animals showing adverse effects	LC 100 = ≥ 10 mg a.i./l 72 h-LC0 = ≤ 2.7 mg/l
4.1.3	Nature of adverse effects	based on mortality
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	6.1, 3.9, 2.5, 1.5 and 1.1 mg tebuconazole / l (measured concentrations)
4.2.2	Actual concentrations of test substance	Measurements were performed two days before the test to establish sufficient a.i. concentrations during the test. In addition of all test solutions at 0, 48 and 96 hours of the definitive exposure samples were taken and analyzed. A method validation/recovery study conducted prior to the initiation of the 96 hour test established an average recovery of tebuconazole from water equal to 102 ± 4.79%.
4.2.3	Effect data (Mortality)	For mortality data as absolute numbers of immobile fish and as percent of exposed animals see table A7_4_1_1-6); LC_{50} = 4.4 mg/l (see also table A7_4_1_1-7)
4.2.4	Concentration / response curve	No graph of the concentration-mortality curve at test termination is in the report

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1

4.2.5	Other effects	At 6.1 mg/l and 3.9 mg/l the surviving fishes show loss of equilibrium and extended (sometimes abrupted) abdomen. At 2.5 mg/l only after 96 hours exposure one fish exhibited a partial loss of equilibrium and one exhibited darkened pigmentation
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	No animals in the controls exhibited adverse effects
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 flow through test was performed according to the EPA guidelines. The authors report some minor deviations from the protocols mentioned under 2.1 (e.g. feeding during 48 hours before the test, slightly minor water hardness). These are regarded as not relevant for the test result (for more details see report page 14).
5.2	**Results and discussion**	A LC 50 of 4.4 mg a.i./l was shown from the test based of measured tebuconazole concentrations. The measured concentrations show no relevant deviations during the exposure time. No mortalities were found in the controls.
5.2.1	96h-LC_0	1.5 mg/l
5.2.2	96h-LC_{50}	4.4 mg/l
5.2.3	96h-LC_{100}	> 6.1 mg/l
5.3	**Conclusion**	The OECD validity criteria can be considered as fulfilled (see validity criteria summarized in table A7_4_1_1-8). A dose-response relationship can be seen from the experiments.
5.3.1	Other conclusions	-
5.3.2	Reliability	[redacted]
5.3.3	Deficiencies	[redacted]

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*02 04 04*
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	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*
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, acetone
Concentration of vehicle	(% v/v)
Vehicle control performed	Yes, duplicate
Other procedures	Flow through system, sampling for a.i. concentration measurements by HPLC at 0, 48 h and 96 h exposure time

Table A7_4_1_1-2: Dilution water

Criteria	Details
Source er	Well water
Alkalinity	33 mg/l
Hardness	30 mg/l
pH	7.1
Oxygen content	79-80%
Conductance	110 µmho / cm
Holding water different from dilution water	No

Table A7_4_1_1-3: Test organisms

Criteria	Details
Species/strain	*Oncorhynchus mykiss*(rainbow trout – *Salmo gaidneri*)
Source	[redacted]
Wild caught	No
Age/size	30-38 cm
Kind of food	Pelleted commercial food
Amount of food	*Ad libitum*
Feeding frequency	Daily
Pretreatment	14 days acclimation period
Feeding of animals during test	No

Table A7_4_1_1-4: Test system

Criteria	Details
Test type	Flow-through
Renewal of test solution	6.5 volume replacement per 24 hour
Volume of test vessels	29 x 20 x 25 centimetres (each filled with 15 l water)
Volume/animal	0.19 biomass / l
Number of animals/vessel	10
Number of vessels/ concentration	2
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_1-5: Test conditions

Criteria	Details
Test temperature	12 +-1 °C
Dissolved oxygen	8.8 – 9.8 mg/l (control 9.0-9.5 mg/l)
pH	6.9-7.5
Adjustment of pH	No
Aeration of dilution water	No
Intensity of irradiation	16-36 footcandle (= 172 – 378 lux per m²) at water surface
Photoperiod	16 h photoperiod daily

Table A7_4_1_1-6: Mortality data

Test Substance Measured Concentration [mg/l]	Mortality Results from Duplicate Samples							
	Number				Percentage			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
6,1	[redacted]							
3,9	[redacted]							
2,5	[redacted]							
1,5	[redacted]							
1,0	[redacted]							
Control	[redacted]							
Control with solvent	[redacted]							
Temperature [°C]	12- 13							
pH	6.9 – 7.5							
Oxygen [mg/l]	8.8 – 9.8 (control 9.0 – 9.7)							

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	[redacted]		[redacted]	
LC_{50}	[redacted]		[redacted]	[redacted]
LC_{100}	[redacted]		[redacted]	

[1] indicated if effect data are based on nominal (n) or measured (m) 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%	yes	
Concentration of dissolved oxygen in all test vessels > 60% saturation	yes	
Concentration of test substance ≥80% of initial concentration during test	yes	

Criteria for poorly soluble test substances	not applicable	

Section A7.4.1.1 Acute toxicity of 1,2,4-triazole on rainbow trout

Annex Point IIA7.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Report on the test for acute toxicity of CGA 98032 to rainbow trout. [redacted]; unpublished report No. 821418; date: 1983-08-30	
1.2	**Data protection**	[redacted]	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD 203 (1981)	
2.2	**GLP**	[redacted]	
2.3	**Deviations**	[redacted]	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2,4-triazole	
3.1.1	Lot/Batch number	batch No. EN38530	
3.1.2	Specification	Technical material	
3.1.3	Purity	purity [redacted]	
3.1.4	Composition of Product	n.a.	
3.1.5	Further relevant properties	-	
3.1.6	Method of analysis	Nominal concentrations, GC analysis at time 0 and after 96 hours	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	n.a.	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	n.a.	

Section A7.4.1.1 Acute toxicity of 1,2,4-triazole on rainbow trout

Annex Point IIA7.1

3.4	**Testing procedure**	Juvenile rainbow trout, *Oncorhynchus mykiss* (mean body length 53 mm; mean body weight 1.27 g), were exposed to nominal levels of 100, 180, 320, 580 and 1000 mg 1,2,4-triazole/L in a static system for 96 hours. Dechlorinated tap water was used to prepare the test solutions. The test incorporated two replicate tanks of five fish for each exposure concentration and for the untreated water control. Mortalities and symptoms of toxicity were recorded at intervals of 24 hours throughout the test up to 98 hours. Dissolved oxygen, temperature and pH were recorded during the study and the concentrations of 1,2,4-triazole in the test systems were determined by gas chromatography (GC) at 0 and 96 hours Over the test period water temperature was maintained at 15 °C, pH ranged between 7.6 - 8.1 and dissolved oxygen concentrations ranged between 8.2 - 10.1 mg/L. The test concentrations of 1,2,4-triazole ranged from 55 to 86% of nominal at study start and from 52 to 73% of nominal in the samples taken after 96 hours.
		4 RESULTS
4.1	**Limit Test**	Not performed
4.2	**Results test substance**	
		The results based on both are summarised in table7_4_1_1-1
4.2.1	Other effects	Over the exposure period, abnormal swimming behavior and loss of equilibrium was observed in fish exposed to 100 (52), 320 (192) and 580 (378) mg 1,2,4-triazole/L and there were slight effects on pigmentation in the 580 (378) mg/L treatment group (mean measured concentrations in brackets).
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	0 %
4.3.2	Nature of adverse effects	n.a.
4.4	**Test with reference substance**	Not performed

Section A7.4.1.1 Acute toxicity of 1,2,4-triazole on rainbow trout

Annex Point IIA7.1

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The test was performed according to OECD 203 (1981). Juvenile rainbow trout, *Oncorhynchus mykiss* were exposed to nominal levels of 100, 180, 320, 580 and 1000 mg 1,2,4-triazole/L in a static system for 96 hours. Mortalities and symptoms of toxicity were recorded at intervals of 24 hours throughout the test up to 98 hours. Dissolved oxygen, temperature and pH were recorded during the study and the concentrations of 1,2,4-triazole in the test systems were determined by gas chromatography (GC) at 0 and 96 hours. The test concentrations of 1,2,4-triazole ranged from 55 to 86% of nominal at study start and from 52 to 73% of nominal in the samples taken after 96 hours.
5.2	**Results and discussion**	The results are summarized in table 7.4.1.1-1. Over the exposure period, abnormal swimming behavior and loss of equilibrium was observed in fish exposed to 52, 192 and 378 mg 1,2,4-triazole/L (based on mean measured concentrations).
5.2.1	LC_0	378 mg/L based on a measured concentration
5.2.2	LC_{50}	498 mg/L extrapolated, based on measured concentrations
5.2.3	LC_{100}	657 mg/L based on a measured concentration
5.3	**Conclusion**	Based on mean measured concentrations, the 96 h-LC_{50} for 1,2,4-triazole in rainbow trout was 498 mg/L.
5.3.1	Other conclusions	1,2,4-triazole has no significant toxicity against rainbow trout
5.3.2	Reliability	[redacted]
5.3.2	Deficiencies	[redacted]

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*April 07*
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	

Section A7.4.1.1 Acute toxicity of 1,2,4-triazole on rainbow trout

Annex Point IIA7.1

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

Table A7_4_1_1-1: Results of the acute fish test according to OECD Guideline 203

		Mortality [%]			
Nominal	**Mean measured**	**24h**	**48h**	**72h**	**96h**
Water	-				
100	52				
180	132				
320	192				
580	378				
1000	657				
LC_{50} [mg/L] based on nominal concentrations		> 1000	800	760	760
LC_{50} [mg/L] based on mean measured concentrations (confidence intervals $p \leq 0.05$)		> 657	528 (n. d.)	498 (378 - 657)	498 (378 - 657)

n.d = non detectable

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 *Daphnia magna*

Official use only

		1 REFERENCE
1.1	**Reference**	Forbis A.D, 1988, Acute Flow-Through of HWG-1608 to *Daphnia magna,* ABC Laboratories, Inc. No. 96791.
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes OECD No.202 U.S.-EPA-Fifra, 40 CFR, Sec. 158.145 Guideline 72-2
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]
		3 MATERIALS AND METHODS
3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	batch number [redacted]
3.1.2	Specification	As given in section 2
3.1.3	Purity	[redacted] of active substance
3.1.4	Composition of Product	
3.1.5	Further relevant properties	
3.1.6	Method of analysis	HPLC by direct aqueous injections. See standard format A4
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**	

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 *Daphnia magna*

3.4.1	Dilution water	see table A7_4_1_2-2
3.4.2	Test organisms	see table A7_4_1_2-3
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 e.g. immobility
3.4.7	Sampling	0, 24 and 48 hours. Chemical analysis at 0 and 48 hours
3.4.8	Monitoring of TS concentration	Yes 0 and 48 hours
3.4.9	Statistics	Statistical analysis was obtained by employing a computerized program using the binominal, the moving averange, and the probit tests.

4 RESULTS

4.1	**Limit Test**	Performed						
4.1.1	Concentration	0.1, 1.0, 2.0, 5.0, 7.0 and 10.0 mg/l						
4.1.2	Number/ percentage of animals showing adverse effects	Concentration (mg/l):	0.1	1.0	2.0	5.0	7.0	10.0
		Mortality (in %)	0	0	20	50	40	100
4.1.3	Nature of adverse effects	Mortality						
4.2	**Results test substance**							
4.2.1	Initial concentrations of test substance	0.46, 0.74, 1.6, 2.6, and 6.2						
4.2.2	Actual concentrations of test substance	Same as 4.2.1						
4.2.3	Effect data (Immobilisation)	see table A7_4_1_2-7						
4.2.4	Concentration / response curve	Dose-response Slope = 3.8						
4.2.5	Other effects	The abnormal effects of mortality, quiescence, and/or daphnids lying on the bottom of test vessels were observed in 1.6, 2.6 and 6.2 mg/l test concentrations						
4.3	**Results of controls**	No mortality occurred in the control treatments						
4.4	**Test with reference**	Not performed						

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 *Daphnia magna*

	substance	
4.4.1	Concentrations	
4.4.2	Results	
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The test is performed in a flow-through system and is according to OECD No. 202 and no relevant deviations from test guidelines is seen
5.2	**Results and discussion**	
5.2.1	96h-LC_0	2.6 mg/l after 24 h and 0.74 after 48 h
5.2.2	96h-LC_{50}	> 6.2 mg/l after 24 h, and 4.2 mg/l after 48 h
5.2.3	96h-LC_{100}	> 6.2 mg/l
5.3	**Conclusion**	The test is valid and performed according to OECD 202 Acute Immobilisation test. It must however, be noted that the report has calculated the LC50 value instead of the EC50 value. Therefore the EC50 value based on immobilisation is lower than 4.2 after 48 hours
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	[redacted]

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 *Daphnia magna*

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*02 04 04*
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
	[redacted]
Remarks	[redacted]
	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*
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 devise
Vehicle	Yes Dimethylformamide (DMF)
Concentration of vehicle	
Vehicle control performed	Yes The solvent control received an aliquot (0.050 ml) of dimethylformamide which was equivalent to that of the other treatment chambers.
Other procedures	

Table A7_4_1_2-2: Dilution water

Criteria	Details
Source	Aerated, aged ABC well water
Alkalinity ($CaCO_3$)	304 – 312 mg/l
Hardness ($CaCO_3$)	224 mg/l
pH	7.8 – 8.3
Ca / Mg ratio	
Na / K ratio	
Oxygen content	9.2- 10.1 ppm
Conductance	
Holding water different from dilution water	

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)	$\leq$24 – hours old
Breeding method	
Kind of food	Algae (*Selenastrum capricornutum*) supplemented with a suspension of Tetramin, yeast and mixed cereal leaves.
Amount of food	
Feeding frequency	
Pretreatment	
Feeding of animals during test	No

Table A7_4_1_2-4: Test system

Criteria	Details
Renewal of test solution	Flow-through system and aerated, aged ABC well water was delivered to each test chamber at a rate of 4.1 ml/minute, an amount which was sufficient to replace the 1-liter test volume approximately 6.0 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 replicates, 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 °C +/- 2 °C
Dissolved oxygen	8.4 mg/l
pH	8.3
Adjustment of pH	No
Aeration of dilution water	Yes
Quality/Intensity of irradiation	e.g. white-type fluorescent lamps; light-temperature 4200 K; 30 - 100 lm at water surface
Photoperiod	e.g. 16 h daylight photoperiod

Table A7_4_1_2-6: Immobilisation data

Test-Substance Concentration (effective) [1] [mg/l]	Immobile *Daphnia*						
	Number		Percentage		Oxygen [mg/l]	pH	Tempera-ture [°C]
	24 h	48 h	24 h	48 h	48 h	48 h	48 h
Control	[redacted]				8.4	8.4	20
Solvent control	[redacted]						20
0.46	[redacted]				8.4	8.3	20
0.74	[redacted]						20
1.6	[redacted]				8.4	8.3	20
2.6	[redacted]						20
6.2	[redacted]				8.4	8.3	20

[1] TS concentrations were mean measured

Table A7_4_1_2-7: Effect data

	EC_{50} [1]	95 % c.l.	EC_0 [1]	EC_{100} [1]
24 h [mg/l]	*> 6.2 (m)*	Could not be calculated	2.6 (m)	> 6.2
48 h [mg/l]	4.2 (m)[2]	3.6 – 4.9 [2]	0.74 (m)	> 6.2

[1] indicate if effect data are based on nominal (n) or measured (m) concentrations

[2] 4.2 mg/l is based on motility, LC50 and not EC50

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		

Section A7.4.1.2 Acute toxicity of 1,2,4-triazole to daphnia magna

Annex Point IIA7.2

Official use only

	1 REFERENCE	
1.1	**Reference**	Bell, G.: Fluqinconazole, technical material, 100.8% w/w - 1,2,4-triazole: acute toxicity to *Daphnia magna*, Huntingdon Life Sciences, Huntingdon, UK, TDMG; unpublished report No. ENVIR/95/52; date: 1995-11-29
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD 202. Directive 92/69/EEC, Method C.2
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]
	3 MATERIALS AND METHODS	
3.1	**Test material**	1,2,4-triazole
3.1.1	Lot/Batch number	batch No. [redacted], purity [redacted]
3.1.2	Specification	Pure substance
3.1.3	Purity	[redacted]
3.1.4	Composition of Product	n.a.
3.1.5	Further relevant properties	-
3.1.6	Method of analysis	Nominal concentrations, verification by gas-liquid chromatography
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	n.a.
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	n.a.

Section A7.4.1.2 Acute toxicity of 1,2,4-triazole to daphnia magna

Annex Point IIA7.2

3.4	**Testing procedure**	
		The potential toxicity of 1,2,4 triazole to Daphnia magna was investigated in a preliminary range-finding test followed by a definitive limit test. In the range-finding test, the test substance was dispersed directly into dilution water, without the use of auxiliary solvents, to produce nominal concentrations of 0.1, 1, 10 and 100 mg/L. The test incorporated two vessels for each concentration level and for a control treatment of dilution water. Ten first instar Daphnia were introduced into each test vessel and maintained for 48 hours. Numbers of immobilized Daphnia were recorded after 24 and 48 hours. In the definitive test, a single concentration of 100 mg/L was prepared was direct addition of the test substance to the dilution water. The test incorporated four replicate cultures for the exposure concentration and the control treatment of dilution water. Five daphnids were introduced to each exposure vessel and monitored at 24 and 48 hours for immobilization. Temperature, pH and dissolved oxygen were recorded at 0 and 48 hours. Water samples were collected at 0 and 48 hours for analysis of test substance concentration. In the definitive test, water temperature, pH and dissolved oxygen concentration ranged between 21 – 22 °C, 7.6 - 7.9, and 7.7 - 8.4, respectively, over the test duration. Measured concentrations of 1,2,4-triazole varied from 94 % of nominal at the test start to 102 % of nominal at the test termination.
		4 RESULTS
4.1	**Limit Test**	See "definitive test"
4.2	**Results test substance**	
		The results based on both are summarised in table 7_4_1_2-1
4.2.1	Other effects	No other effects were observed
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	0 %
4.3.2	Nature of adverse effects	n.a.
4.4	**Test with reference substance**	Not performed

Section A7.4.1.2 Acute toxicity of 1,2,4-triazole to daphnia magna

Annex Point IIA7.2

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	After a range finder test (1- 100mg/L) in a limit test a single concentration of 100 mg/L was prepared by direct addition of the test substance to the dilution water. The test incorporated four replicate cultures for the exposure concentration and the control treatment of dilution water. Five daphnids were introduced to each exposure vessel and monitored at 24 and 48 hours for immobilization. Temperature, pH and dissolved oxygen were recorded at 0 and 48 hours. Water samples were collected at 0 and 48 hours for analysis of test substance concentration. Measured concentrations of 1,2,4-triazole varied from 94 % of nominal at the test start to 102 % of nominal at the test termination.
5.2	**Results and discussion**	The results are summarized in table 7.4.1.2-1. No toxic effects were observed.
5.2.1	96h-LC_0	100 mg/L
5.2.2	96h-LC_{50}	> 100 mg/L
5.2.3	96h-LC_{100}	n.a. (limit test at 100 mg/L)
5.3	**Conclusion**	Based on nominal concentrations, the 48-hour EC_{50} for 1,2,4-triazole in Daphnia magna was determined to be > 100 mg/L.
5.3.1	Other Conclusions	1,2,4-triazole has no significant toxicity against daphnia magna
5.3.2	Reliability	[redacted]
5.3.3	Deficiencies	[redacted]

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*April 07*
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	

Section A7.4.1.2 Acute toxicity of 1,2,4-triazole to daphnia magna

Annex Point IIA7.2

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

Table 7_4_1_2-1: Toxicity of 1,2,4-triazole to water fleas (based on nominal concentrations)

Test object	*Daphnia magna*	
Test substance	1,2,4-triazole	
Exposure	24 h, static	48 h, static
EC_{50} [mg/L]	> 100	> 100
NOEC [mg/L]	100	100

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

Official use only

1 REFERENCE

1.1	**Reference**	Heimbach, F. (1987). Growth inhibition of green algae (*Scenedesmus subspicatus*) caused by HWG 1608 (technical), Bayer AG,.
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes OECD No 201 ISO-Guideline ISO/TC 147/SC 5/WG 5 N 84 (Algal Growth Inhibition test)
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	batch number [redacted]
3.1.2	Specification	As given in section 2
3.1.3	Purity	[redacted]
3.1.4	Composition of Product	
3.1.5	Further relevant properties	
3.1.6	Method of analysis	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	see table A7_4_1_3-1
3.3	**Reference substance**	Yes $K_2Cr_2O_7$
3.3.1	Method of analysis for reference substance	

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

3.4	**Testing procedure**	
3.4.1	Culture medium	The test suspensions contain the following (based on 1 litre): 15 mg NH_4Cl, 12 mg $MgCl_2$ x$6H_2O$, 18 mg $CaCl_2$ x$2H_2O$, 15 mg $MgSO_4$ x$7H_2O$, 1.6 mg KH_2PO_4, 80 µg $FeCl_3$ x$6H_2O$, 100µg Na_2EDTA x$2H_2O$, 185 µg H_3BO_3, 415 µg$MnCl_2$ x$4H_2O$, 3 µg $ZnCl_2$, 1.5µg $CoCl_2$ x$6H_2O$, 0.01 µg $CuCl_2$ x$2H_2O$, 7µgNa_2MoO_4 x$2H_2O$ and 50 mg $NaHCO_3$. pH = 8.15
3.4.2	Test organisms	see table A7_4_1_3-2
3.4.3	Test system	see table A7_4_1_3-3
3.4.4	Test conditions	see table A7_4_1_3-4 The cell counts are obtained indirectly by a photometric measurement of the extinction/turbidity. These measurements are then calibrated with microscopic determination of the cell density.
3.4.5	Duration of the test	
3.4.6	Test parameter	cell multiplication inhibition
3.4.7	Sampling	Sampling intervals: 0, 24, 48. 72, and after 96 hours
3.4.8	Monitoring of TS concentration	No
3.4.9	Statistics	Calculation were performed with a probit analysis according to the "maximum likelihood" method
		4 RESULTS
4.1	**Limit Test**	Performed
4.1.1	Concentration	Control, solvent control (acetone 0.1 ml/l), 0.01, 0.1, 1.0, and 10 mg a.i./l
4.1.2	Number/ percentage of animals showing adverse effects	An inhibition of cell proliferation was seen at concentrations above 0.1 mg/l and a complete inhibition was found at 10 mg/l.
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	Nominal concentrations: control, solvent control (acetone 0.1 ml/l), 0.32, 0.56, 1.0, 1.8, 3.2, 5.6, and 10.0 mg a.i./l
4.2.2	Actual concentrations of test substance	No measurements conducted during test
4.2.3	Growth curves	
4.2.4	Concentration / response curve	

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

4.2.5	Cell concentration data	see table A7_4_1_3-5
4.2.6	Effect data (cell multiplication inhibition)	The EC50 of the biomass growth (E_bC_{50}) after 72 hours was 1.96 mg a.i./l and after 96 hours 1.64 mg/l. The EC50 of the growth rate (E_rC_{50}) after72 hours was 5.3 mg a.i./l and after 96 hours 4.01 mg a.i./l. The no-observed- effect-concentration (NOEC) was 0.32 a.i./l (biomass) and 1.0 mg a.i./l (growth rate). The lowest concentration tested with signs of toxicity was 0.56 mg a.i./l (biomass) and 1.8 mg a.i./l (growth rate).
4.2.7	Other observed effects	No
4.3	**Results of controls**	Mean cell density cells x 10^4 /ml

0 hours	1
24 hours	6.28
48 hours	26.95
72 hours	98.32
96 hours	228.59

4.4	**Test with reference substance**	Performed
4.4.1	Concentrations	Control, 0.18, 0.32, 0.56, 1.0, 1.8 mg/l
4.4.2	Results	EC50 based on biomass after 96 hours = 0.44 mg/l and EC50 based on growth rate = 1.20 mg/l

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Growth inhibition test on algae according to OECD 201. The test was prolonged to 96 hours. No significant derivations from the guideline
5.2	**Results and discussion**	The growth rate in the control vessels after 3 days is greater than the factor 16 and therefore fulfils the quality criteria of the ISO and OECD guideline.
5.2.1	NOErC	1.0 mg a.i./l for (growth rate) and 0.32 mg a.i./l for (biomass)
5.2.2	ErC_{50}	5.30 mg a.i./l after 72 hours and 4.01 mg/l after 96 hours
5.2.3	EbC_{50}	1.96 mg a.i./l after 72 hours and 1.64 mg/l after 96 hours
5.3	**Conclusion**	Validity criteria can be considered as fulfilled. There is a clear dose-response relationship and a complete inhibition was found at 10 mg/l. Tebuconazole is toxic to algae.
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	[redacted]

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	02 04 04
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	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*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_4_1_3-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes Ultrasonic bath for 30 min
Vehicle	Yes Acetone
Concentration of vehicle	0.01% v/v
Vehicle control performed	Yes growth inhibition test
Other procedures	No

Table A7_4_1_3-2: Test organisms

Criteria	Details
Species	Green algae *Scenedesmus subspicatus*
Strain	*Scenedesmus subspicatus* strain SAG 86/81
Source	
Laboratory culture	Yes
Method of cultivation	Under sterile conditions
Pretreatment	
Initial cell concentration	10^4 cells/ml exponentially grown *Scenedesmus subspicatus*

Table A7_4_1_3-3: Test system

Criteria	Details
Volume of culture flasks	300 ml Erlenmeyer flasks
Culturing apparatus	Controlled environment cabinet
Light quality	2 x 4 fluorescent lamps
Procedure for suspending algae	Shaking
Number of vessels/ concentration	3
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_3-4: Test conditions

Criteria	Details
Test temperature	23
pH	8.15
Aeration of dilution water	?
Light intensity	8000 Lux constant light
Photoperiod	Constant light

Table A7_4_1_3-5: Cell concentration data

Test-Substance Concentration (nominal/effective)[1] [mg/l]	Cell concentrations (mean values) [cells/ml]							
	measured				Percent of control			
	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
control	[redacted]							
Solvent control	[redacted]							
0.32	[redacted]							
0.56	[redacted]							
1.0	[redacted]							
1.8	[redacted]							
3.2	[redacted]							
5.6	[redacted]							
10.0	[redacted]							
Temperature [°C]	23	23	23	23				
pH	8.09	7.96	8.40	8.14				

[1] specify, if TS concentrations were nominal or measured

Table A7_4_1_3-6: Validity criteria for algal growth inhibition test according to OECD Guideline 201

	fulfilled	Not fulfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	X	
Concentration of test substance ≥80% of initial concentration during test		X

Criteria for poorly soluble test substances		

Section A7.4.1.3 — Acute toxicity of 1,2,4-triazole to *Selenastrum capricornutum* (new name: *Pseudokirchneriella subcapitata*)

Annex Point IIA7.3

Official use only

1 REFERENCE

1.1	**Reference**	Palmer S.J., Kendall T.Z. and Krueger H.O.: 1,2,4-triazole: A 96-hours toxicity test with the freshwater alga (Selenastrum capricornutum). Source: Wildlife International Ltd, USA; unpublished report No. 528A-101; date: 2001-08-31
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes, OECD 201; EU Directive 92/69/EEC, Method C.3; U.S. EPA OPPTS Number 850.5400
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

3.1	**Test material**	1,2,4-triazole
3.1.1	Lot/Batch number	Batch No. [redacted]
3.1.2	Specification	Technical 1,2,4-triazole
3.1.3	Purity	[redacted]
3.1.4	Composition of Product	n.a.
3.1.5	Further relevant properties	-
3.1.6	Method of analysis	Nominal concentrations and analytical controls
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	n.a.
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	n.a.

Section A7.4.1.3 Acute toxicity of 1,2,4-triazole to *Selenastrum capricornutum* (new name: *Pseudokirchneriella subcapitata*)

Annex Point IIA7.3

3.4	**Testing procedure**	Three replicate cultures, containing 1 x 104 cells/ml of the freshwater green alga Pseudokirchneriella subcapitata (formerly Selenastrum capricornutum), were exposed to nominal concentrations of 1.9, 3.8, 7.5, 15 and 30 mg 1,2,4-triazole/L plus a negative control (pure culture medium) under static conditions for 96 hours. One additional replicate was maintained in each control and treatment group to provide test solution for verification of the test substance concentration at 72 and 96 hours. Algae were maintained at 23 □□ 2 °C with continuous cool-white fluorescent lighting of 6500 lux □□ 10 %. pH was measured at 0 and 96 hours. Algal samples were collected from each test culture at 24-hour intervals to determine cell densities. Cell densities, areas under the growth curve (biomass) and growth rates were used to calculate percent inhibition values relative to the control. During the test period, temperatures ranged from 22.0 to 24.3 oC. pH in the test systems was 8.0 at 0 hours and ranged between 8.1 and 9.7 at 96 hours. Concentrations of 1,2,4-triazole ranged from 86 to 101 % of nominal at 0 hours and from 78 to 103 % of nominal at 96 hours.
	4 RESULTS	
4.1	**Limit Test**	Not performed
4.2	**Results test substance**	The tests were performed taking into account different endpoints. Table 7_4_1_3-1 show the results respect to the endpoint cell density, 7_4_1_3-2 show the results respect to the endpoint biomass, 7_4_1_3-3 show the results respect to the endpoint growth rate, 7_4_1_3-4 summarises all endpoints. EC 50 based on the growth rate: > 31 mg/L EC 50 based on the biomass: 13 mg/L EC 50 based on the cell density: 12 mg/L
4.2.1	Other observed effects	-
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	No adverse effects were observed
4.3.2	Nature of adverse effects	n.a.
4.4	**Test with reference substance**	Not performed

Section A7.4.1.3 Acute toxicity of 1,2,4-triazole to *Selenastrum capricornutum* (new name: *Pseudokirchneriella subcapitata*)

Annex Point IIA7.3

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Three replicate cultures, containing 1 x 10^4 cells/ml of the freshwater green alga *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*), were exposed to nominal concentrations of 1.9, 3.8, 7.5, 15 and 30 mg 1,2,4-triazole/L plus a negative control (pure culture medium) under static conditions for 96 hours. One additional replicate was maintained in each control and treatment group to provide test solution for verification of the test substance concentration at 72 and 96 hours.
5.2	**Results and discussion**	Results are based on n measured concentrations and summarized in table 7_4_1_3_4.
5.2.1	NOErC	Not determined
5.2.2	EC_{50}	> 31 mg/L based on growth rate, 12 mg/L based on the cell density and 13 mg/L based on the biomass.
5.2.3	EC_{100}	Not determined
5.3	**Conclusion**	
5.3.1	Other Conclusions	
5.3.2	Reliability	[redacted]
5.3.3	Deficiencies	[redacted]

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*April 07*
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	

Section A7.4.1.3 — Acute toxicity of 1,2,4-triazole to *Selenastrum capricornutum* (new name: *Pseudokirchneriella subcapitata*)

Annex Point IIA7.3

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

Table 7_4_1_3-1: Effect of 1,2,4-triazole on algal cell density

Test object	*Pseudokirchneriella subcapitata* (*Selenastrum capricornutum*)			
Test substance	1,2,4-Triazole, *M26*			
Mean measured concentration [mg/L]	Mean cell density (% of control)			
	24 hours	48 hours	72 hours	96 hours
Control	0-	0	0	0
1.7	[redacted]	[redacted]	[redacted]	[redacted]
3.1	[redacted]	[redacted]	[redacted]	[redacted]
6.8	[redacted]	[redacted]	[redacted]	[redacted]
14	[redacted]	[redacted]	[redacted]	[redacted]
31	[redacted]	[redacted]	[redacted]	[redacted]

* significantly different from control ($p < 0.05$)

Table 7_4_1_3-2: Effect of 1,2,4-triazole on algal biomass

Test object	*Pseudokirchneriella subcapitata* (*Selenastrum capricornutum*)			
Test substance	1,2,4-Triazole, *M26*			
Mean measured concentration [mg/L]	Mean biomass (% of control)			
	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0
1.7	[redacted]	[redacted]	[redacted]	[redacted]
3.1	[redacted]	[redacted]	[redacted]	[redacted]
6.8	[redacted]	[redacted]	[redacted]	[redacted]
14	[redacted]	[redacted]	[redacted]	[redacted]
31	[redacted]	[redacted]	[redacted]	[redacted]

*significantly different from control ($p < 0.05$)

Table 7_4_1_3_3: Effect of 1,2,4-triazole on algal growth rate

Test object	*Pseudokirchneriella subcapitata* (*Selenastrum capricornutum*)			
Test substance	1,2,4-Triazole, *M26*			
Mean measured concentration [mg/L]	Growth rate (% of control)			
	24 hours	48 hours	72 hours	96 hours
Control	-	-	-	-
1.7	[redacted]	[redacted]	[redacted]	[redacted]
3.1	[redacted]	[redacted]	[redacted]	[redacted]
6.8	[redacted]	[redacted]	[redacted]	[redacted]
14	[redacted]	[redacted]	[redacted]	[redacted]
31	[redacted]	[redacted]	[redacted]	[redacted]

* significantly different from control ($p < 0.05$)

Table 7_4_1_3-4: Summary of EC_{50} values over the 96-hour exposure period

Test object	*Pseudokirchneriella subcapitata* (*Selenastrum capricornutum*)					
Test substance	1,2,4-Triazole, *M26*					
Time	Cell density		Biomass		Growth rate	
	EC_{50} [mg/L]	95 % CI [mg/L]	EC_{50} [mg/L]	95 % CI [mg/L]	EC_{50} [mg/L]	95 % CI [mg/L]
24 Hours	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
48 Hours	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
72 Hours	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
96 Hours	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

n. d.: 95 % confidence interval could not be determined. CI: confidence limit

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

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		1 REFERENCE
1.1	**Reference**	Dr. G. Mueller (study director), 1993, BAYER AG Institute for Environmental Analysis and Assessments, Test No. 419 A/93
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	ISO Guideline 8192-1986 (E) which largely corresponds to OECD 209
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]
		3 MATERIALS AND METHODS
3.1	**Test material**	Tebuconazole (product number [redacted])
3.1.1	Lot/Batch number	Batch number [redacted]
3.1.2	Specification	Technical grade
3.1.3	Purity	[redacted]
3.1.4	Composition of Product	
3.1.5	Further relevant properties	Stable in water, not volatile, water solubility: 32 mg/l
3.1.6	Method of analysis	Direct weighting and measurement of oxygen consumption
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not included in the report (dispersion assumed)
3.3	**Reference substance**	Yes - 3,5-Dichlorophenole
3.3.1	Method of analysis for reference substance	Measurement of oxygen consumption
3.4	**Testing procedure**	
3.4.1	Culture medium	Stock solution containing peptone, meat extract urea and mineral salts according to OECD 209

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

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3.4.2	Inoculum / test organism	Activated sludge from a laboratory waste water treatment plant (OECD) without pre-treatment
3.4.3	Test system	BOD flasks with oxygen meter
3.4.4	Test conditions	20 ± 1 °C (see addendum), pH not included in the report (pH according to ISO/OECD 209 = 7.5 ± 0.5)
3.4.5	Duration of the test	30 minutes (see addendum)
3.4.6	Test parameter	Respiration inhibition
3.4.7	Analytical parameter	Oxygen measurement
3.4.8	Sampling	
3.4.9	Monitoring of TS concentration	No
3.4.10	Controls	An abiotic oxygen consumption control was performed. Applying a test concentration of 10000 mg/l tebuconazole during 10 minutes no oxygen was consumed. Therefore the test substance shows no abiotic oxygen consumption.
3.4.11	Statistics	Not applicable
		4 RESULTS
4.1	**Preliminary test**	Not performed
4.1.1	Concentration	
4.1.2	Effect data	
4.2	**Results test substance**	
4.2.1	Concentration versus effects on oxygen inhibition	See **table** below
4.2.2	Actual concentrations of test substance	Nominal concentrations
4.2.3	Growth curves	Not reported/not relevant
4.2.4	Cell concentration data	Not reported/not relevant
4.2.5	Concentration/ response curve	Not provided /not relevant (see table)
4.2.6	Effect data	E_{50} = > 10 000 mg/l
4.2.7	Other observed effects	No other effects observed
4.3	**Results of controls**	

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

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4.4	**Test with reference substance**	Performed with 3,5-Dichlorophenol
4.4.1	Concentrations	1 and 20 mg/l
4.4.2	Results	EC50: 9.6 mg/l (see addendum)
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The study was carried out under GLP according to ISO8192/OECD209 guideline, SOP were available for all relevant test steps.
5.2	**Results and discussion**	At nominal concentrations of 1000-10000 mg a.s./l [water solubility of tebuconazole = 32 mg/l] inhibition of oxygen formation in activated sludge was observed between 8% and 11%
5.2.1	EC_{20}	> 10 000 mg/l
5.2.2	EC_{50}	> 10 000 mg/l
5.2.3	EC_{80}	> 10 000 mg/l
5.3	**Conclusion**	Up to the limit nominal concentration of test substance only an insignificant inhibition of 11% was observed
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	[redacted]

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*02 04 04*
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

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

Table A7_4_1_4-1: Test Results based on nominal concentrations

Test Compound [mg/l]	Respiratory Rate [mg O_2/l h]	Inhibition [%]
1 000	[redacted]	[redacted]
1 800	[redacted]	[redacted]
3 200	[redacted]	[redacted]
5 600	[redacted]	[redacted]
10 000	[redacted]	[redacted]

Section A7.4.2 Bioconcentration in aquatic organisms (fish)

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1 REFERENCE

1.1	**Reference**	[redacted] 1988, HWG 1608: Bioaccumulation in Fish. [redacted]-Report No. 2932, [redacted] Report No. BF-001, January 22, 1988
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	OECD Guideline 305 E
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

3.1	**Test material**	a) HWG 1608 Phenyl UL-C14-radiolabelled test substance (84.3 µCi/mg) b) Non radioactive test substance
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	a) HWG 1608 Phenyl UL-C14 b) HWG 1608 analytical standard
3.1.3	Purity	a) [redacted] b) [redacted] of active substance
3.1.4	Further relevant properties	none
3.1.5	Radiolabelling	[phenyl-UL-^{14}C]
3.1.6	Method of analysis	Processing of fish samples for radioassay The fish samples taken according to the sampling schedule were collected singly in weighed polystyrene vials suitable for further handling. After determining the wet weight of the samples they were lyophilized, reweighed, and homogenized. Aliquotal parts were taken for combustion followed by liquid scintillation counting (LSC) of the trapped carbon dioxide. Radioactivity measurement was performed in the "[redacted]". At least triplicate subsamples were analysed due to inhomogeneities in the freeze-dried tissues caused mainly by the scales. Extraction of fish samples The lyophilized fish samples (four of each concentration group) were

combined, homogenized and the total radioactivity of the pool determined.

The material was extracted 5 times with 50 ml Acetonitril (ACN), each time, under ultrasonication. The individual solutions were radioassayed, combined and evaporated; The remaining solids were reextracted 5 times with 50 ml Methanol (MeOH), each time, and again 4 times with 50 ml water. The extracted solids were dried. After LSC the MeOH- and the aqueous extracts were pooled, the MeOH evaporated and the remaining aqueous layer freeze-dried. The residue was redissolved in 150 ml MeOH and stirred with 15 g of reversed phase (RP18) material (Lichroprep, Merck), then evaporated. The remaining dry RP material was eluted in a batchwise manner by stirring and filtration 5 times with 50 ml of a water/ACN-mixture and 4 times with pure ACN.

The ACN extract of the lyophilized fish samples and the ACN eluate of the RP18 material were combined for analysis.

Chromatographic methods

For analytical high performance liquid chromatography (HPLC) the following materials and methods were utilized:

- HP 1090 liquid chromatograph equipped with variable wavelength detector or diode array detector HP 1040 (DAD);
- Radioactivity flow through detector Ramona 4 or 6 (Raytest) with solid scintillator cells;
- Lichrosorb RP 8, 5 um, 250 mm long, 4 mm i.d. (Merck)
- Lichrospher 100 RP 18 Super, 250 mm long, 4 mm i.d. (Merck)

The chromatographic conditions utilized were as follows:

flask A: aqueous buffer, pH 7.0

flask B: ACN

time schedules

system 1		system 2	
min	% B	min	% B
0	40	0	5
40	40	45	20
45	100	65	100
75	100	75	100
80	40	80	5

3.2	**Reference substance**	None
3.2.1	Method of analysis for reference substance	No reference substance used
3.3	**Testing/estimation procedure**	
3.3.1	Test system/	Groups of Bluegill sunfish were exposed for 3 days to two different

performance

concentrations of [phenyl-UL-^{14}C]HWG 1608 (0.2 and 0.02 mg/l (nominal)). An additional group was used as untreated control. To measure the elimination of radioactivity from the fish tissue after exposure, the test fish were placed in clean water for 6 days. Fish and water samples were taken 0, 6, 12, 24, 48, 72, 108, 144, 180 and 216 hours after test initiation for survey of radioactivity by liquid scintillation counting. In fish sampled at the end of the exposure period, the nature of the residues was determined (metabolite identification).

Animals

The bluegill sunfish (Lepomis macrochirus) (lot 8/87) used in the bioconcentration study was obtained from [redacted] The fish were identified to species by the supplier. All test fish were held in culture tanks on a l6-hour daylight photoperiod and observed for at least 14 days prior to testing. Fish culture techniques were basically those described by Brauhn et al. (5). Upon arrival a therapeutic disease treatment with tetracycline for fin rot disease was necessary. During the acclimation and test periods, no fish of that lot died. The fish received a standard commercial fish food (Tetra-min, Tetra-Werke) D 4230 Melle, FRG) once daily ad libitum.

Exposure System

A dosing system consisting of a Hamilton Microlab MT dispenser with a 500 ul-syringe for each aquarium controlled by an EPSON HX2O computer (for dosing of stock solution) and flow-meters (for water flow control) was used for the introduction of [phenyl-UL-^{14}C)HWG 1608 and diluent water into the (79 cm x 35 cm x 39 cm) 110 litre test aquaria. Aerated reconstituted water according to OECD Guideline 203 (10) was delivered to the glass aquaria at an average rate of approx. 25 l per hour per aquarium during the exposure period (3 days), an amount which was sufficient to replace the approximately 100 litre test volume approximately 8 times in a 24 hour period and stock solution ([phenyl-UL-^{14}C]HWG 1608 in acetone) was dosed at a rate of 50 ul every 72 seconds (0 2.5 ml/h). Water (continuously 25 l/h) and aliquot of [phenli-UL-^{14}C]HWG 1608 stock solution (2 and 0.2 g/l, 0.05 ml every 72 sec) were delivered to a 2000 ml-mixing cell to yield a nominal exposure concentration of 0.2 and 0.02mg/l, respectively. The mixture was running continuously from the mixing vessel into the respective aquarium. The aquaria were labelled with the study number and the treatment level.

The control aquarium received an amount of acetone solvent equal to that in the exposure aquaria (0.1 ml/l)

The exposure system consisted of one 0.2 mg/l nominal concentration aquarium, one 0.02 mg/l nominal concentration aquarium and one control aquarium.

The test aquaria were arranged in a lab room and constantly kept at 21°C by adding diluent water temperate by an electronically controlled thermostat. Temperature was measured once daily on working days and the range of temperature fluctuations was followed by a mercury-minimum-maximum-thermometer, which was reset after each reading.

The diluter system was calibrated by volumetric measurements of syringe dispenser aliquots and flow-rate of flow meters.

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Preparation of the test substance

The stock solutions for the treatment of the fish were prepared as follows:

140.6 MBq (3.8 mCi) of the radioactive material (ca. 3.12 MBq/mg; 84.3 uCi/mg) were dissolved in 3.8 ml acetone, yielding a solution containing 1 mCi/ml and a concentration of 11.86 mg/ml.

High concentration (0.2 mg/l)

3378 ul of the stock solution (ca. 125 MBq, 40 mg) were added to a solution containing 460 mg non-labelled HWG 1608 in 247 ml acetone to give a final concentration of 2.0 g/l with a specific radioactivity of 250 kBq/mg (corresponding to 21000 dpm per 7-rnl-sample of test water).

Low concentration (0.02 mg/l)

338 ul of the stock solution (ca. 12.5 MBq, 4 mg) were added to a solution containing 46 mg non-labelled HWG 16O8 in 250 ml acetone to give a final concentration of 0.2 g/l with a specific radioactivity of 250 kBq/mg (corresponding to 2100 dpm per 7-ml-sample of test water).

Test procedure

Exposure phase

The exposure phase was initiated by transferring groups of 45 randomly selected and previously acclimatized fish (lot 8/87, length 6.94 ± 0.35 cm, body weight 4.5 ± 0.65 g) to each of the control and test chamber. These fish were observed initially and at least once on working days during the exposure period of 3 days for mortality and/or adverse behaviour. At the same time intervals pH, dissolved oxygen, and nitrite were measured in all aquaria and the temperature in the control tank. Water and fish were sampled throughout the exposure period following the schedules outlined in Table II. Fish were killed by neck dissection, and weighed and processed individually. The water and fish samples were radioassayed and analysed following the procedure outlined in Section 2.3.

Depuration phase

On day 3 of the exposure period, the addition of the [phenyl-UL-^{14}C]HWG 1608 test material was terminated. At the beginning of the depuration phase, the water in the test tanks was removed to a level of ca. 5 cm. Uncontaminated diluent water (21°C) was added at a rate of 25 l/h. The fish were then kept in flowing uncontaminated diluent water (21°C) for 6 days. During the depuration period, water and fish were sampled according to the schedules in Table II and were radioassayed following the procedure in Section 2.3. The fish were observed initially and every 24 hours during the depuration period of 6 days for mortality and/or adverse behaviour. At the same time intervals pH, dissolved oxygen, and nitrite were measured in all aquaria and the temperature in the control tank.

Sampling

Fish

Fish samples were taken during the treatment following the schedule in Table II. On every sampling interval three to ten fish from each chamber were collected and handled separately. The fish were rinsed in uncontaminated water for ca. 3 - 8 sec prior to sacrificing. The fish samples were weighed and either stored frozen in the "[redacted]

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[redacted]" or transferred immediately to the "[redacted]" for further treatment as described in Section 2.3.

At the end of the exposure period four of the ten fish sampled were analyzed for the nature of the residues (identification of metabolites).

Water

At each sampling time, triplicate 7-ml samples were taken directly from each aquarium using polyethylene scintillation counting vials, prepared for control and treated aquaria, and 7-ml aliquots of scintillation cocktail (United Technologies Packard, Instant Scint. Gel) added. The concentration of test substance was calculated by dividing the radioactivity in test water by the specific radioactivity of the test substance, assuming that the radioactivity in the water represented unchanged parent compound.

The concentration of HWG 1608 in water as well as its stability in the stock solutions were not determined.

Stock solutions

The radioactivity concentration of the test substance in both stock solutions was determined by scintillation counting at the end of the test.

Chemical and Physical test parameters

Water quality parameters of temperature, dissolved oxygen, nitrite and pH were measured initially and throughout the study on working days in the control and exposure chambers. The test chambers were not aerated throughout the test. Dissolved oxygen levels remained at or above 94% saturation.

Physical-chemical measurements in the aquaria:

Date			pH-Data Control	0.2 mg/l	0.02 mg/l	Temperature °C
29.09.	Day	0	8.1	8.1	8.1	21
30.09.	Day	1	8.1	8.1	8.1	21
1.10.	Day	2	8.1	8.1	8.1	21
2.10.	Day	3	8.0	8.0	8.0	21
3.10.	Day	4	8.0	8.0	8.0	21
5.10.	Day	6	7.9	7.9	7.9	21
6.10.	Day	7	8.0	8.0	8.0	21
7.10.	Day	8	8.0	8.0	8.0	21
8.10.	Day	9	8.0	8.0	8.0	21
Mean			8.02	8.02	8.02	21
±SD			0.06	0.06	0.06	

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Date			Dissolved oxygen Control mg/l	% sat	0.2 mg/l mg/l	% sat	0.02 mg/l mg/l	% sat.
29.09.	Day	0	8.3	96%	8.2	94%	8.2	94%
30.09.	Day	1	8.5	98%	8.5	98%	8.5	98%
1.10.	Day	2	8.5	98%	8.5	98%	8.5	98%
2.10.	Day	3	8.4	97%	8.4	97%	8.4	97%
3.10.	Day	4	9.0	104%	9.0	104%	9.0	104%
5.10.	Day	6	9.2	106%	9.2	106%	9.2	106%
6.10.	Day	7	9.3	107%	9.3	107%	9.3	107%
7.10.	Day	8	9.2	106%	9.3	107%	9.2	106%
8.10.	Day	9	9.0	104%	9.1	105%	9.0	104%
			8.82	101.6%	8.83	101.8%	8.81	101.5%
			0.37	4.3%	0.41	4.7%	0.39	4.5%

3.3.2 Estimation of bioconcentration

Calculation of results

In evaluating the data obtained from the bioconcentration study, a steady-state approach was used. This consisted of a two compartment model (water and fish) which was used to describe the movement of the test material into and out of the test fish. Thus the steady-state bioconcentration factor (BCF), the uptake rate constant (K1), and the depuration rate constant (K) were determined. To exemplify this, the following reaction is presented:

where:

C_W = concentration of the test material in water, [mg/l])

C_F = concentration of the test material in fish, [mg/kg]

$K_1 = K_u$ = uptake rate constant, [ppm in fish / ppm in water / hour]

$K_2 = K_d$ = depuration rate constant, [1/hour]

The specific radioactivity of HWG 1608 was calculated by the formula:

$$\text{Specific radioactivity (dpm/mg)} = \frac{\text{Total radioactivity in stock solution}}{\text{Total amount of HWG 1608 in stock soln.}}$$

The water concentration of HWG 1608 was calculated by the formula:

Net dpm/ml = radioact. test water – radioact. control water (dpm/ml)

$$\text{Water concentration mg/l} = \frac{\text{(net dpm/ml) * 1000}}{\text{specific activity of parent compound (dpm/mg)}}$$

The tissue concentration of HWG 1608 was calculated by the formula:

Net dpm/g = radioact. test tissue – radioact. control tissue (dpm/g)

$$\text{Tissue concentration mg/kg} = \frac{\text{(net dpm/g) * 1000}}{\text{specific radioact. of parent compound (dpm/mg)}}$$

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Mean values were always calculated using all available digits of the raw data, thus slight inconsistencies in calculating with a limited number of digits are inevitable, but they do not influence the soundness of the final result.

Bioconcentration factors for whole fish during the exposure period were determined by dividing the tissue-radioactivity by the average water-radio-activity (from 0 to sampling time).

dpm/ml (water) = (dpm (treated) - dpm (control))/sample volume

Sample volume in al water radioactivity measurements was 7 ml

$$\text{dpm/g (fish)} = \frac{\text{(dpm/g dry weight (treated) - dpm/q dry weight (control))}}{\text{(sample weight (fresh) / sample weight (dry))}}$$

$$\text{Bioconcentration factor} = \frac{\text{dpm/g (fish)}}{\text{dpm/ml (water)}}$$

The measured values were fitted with a non-linear curve fitting programme using the mean uptake data and the formula:

$$C_F = (K_1/K_2)*C_W*(1-e^{-K_2*t}).$$

With this programme, the calculated bioconcentration factors corresponded well with the observed values.

4 RESULTS

4.1 Experimental data

4.1.1	Mortality/behaviour	no mortalities, no abnormal behaviour
4.1.2	Lipid content	Lipid content not measured, not obligatorily required by OECD 305E

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4.1.3 Concentrations of test material during test

Mean water concentrations: 0.018 ± 0.001 and 0.211 ± 0.003 mg/l, individual values are given in the table below.

Table VI: Radioactivity and concentration of test substance in water (*) mean of all sampling times up to and including that sampling time]

Sample date	dpm water (per 7-ml-sample) Control			Treated			Water dpm/ml Treated - Contr.	Concentr. in water mg/l	dpm/ml water mean 0 - ST*
Exposure 0.2 mg/l (nominal)									
hour 0	8.7	12.8	14.7	22679	22327	22641	3220	0.215	3220
hour 6	6.3	15.0	9.4	21910	21777	22108	3132	0.209	3176
hour 12	15.3	8.2	15.7	22107	22207	21866	3150	0.210	3167
hour 24	9.7	5.6	5.9	22188	22346	21943	3165	0.211	3166
hour 48	7.4	3.7	8.0	22716	22259	22711	3222	0.215	3178
hour 72	10.6	12.3	11.3	22300	21877	21948	3147	0.210	3172
Mean Values			10			22217	3172	0.211	
Standard Deviation			4			309	39	0.003	
Depuration									
hour 108	10.3	12.8	11.2	34	31	41	3	0.00	3172
hour 144	1.3	3.9	6.0	9	8	11	1	0.00	3172
hour 180	6.7	10.3	4.2	34	38	31	4	0.00	3172
hour 216	6.0	8.2	6.9	6	13	10	0	0.00	3172
Mean Values			7			22	2	0.00	
Standard Deviation			3			13	2	0.00	

Sample date	dpm water (per 7-ml-sample) Control			Treated			Water dpm/ml Treated - Contr.	Concentr. in water mg/l	dpm/ml water mean 0 - ST*
Exposure 0.02 mg/l (nominal)									
hour 0	8.7	12.8	14.7	1863	1877	1866	265	0.018	265
hour 6	6.3	15.0	9.4	1811	1839	1883	262	0.017	264
hour 12	15.3	8.2	15.7	1859	1875	1842	264	0.018	264
hour 24	9.7	5.6	5.9	1883	1906	1872	269	0.018	265
hour 48	7.4	3.7	8.0	1937	1963	1951	278	0.019	267
hour 72	10.6	12.3	11.3	1761	1743	1772	250	0.017	264
Mean Values			10			1861	264	0.018	
Standard Deviation			4			61	9	0.001	
Depuration									
hour 108	10.3	12.8	11.2	17	16	27	1	0.000	264
hour 144	1.3	3.9	6.0	4	6	8	0	0.000	264
hour 180	6.7	10.3	4.2	21	10	8	1	0.000	264
hour 216	6.0	8.2	6.9	5	8	6	0	0.000	264
Mean Values			7			11	1	0.000	
Standard Deviation			3			7	1	0.000	

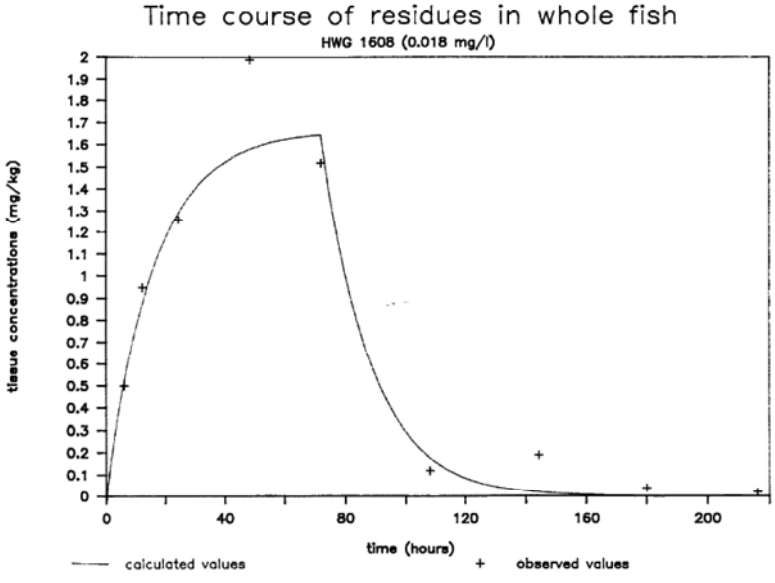
Concentration of test material (with standard deviation and range) for all sampling times in test organisms (total):

Section A7.4.2 Bioconcentration in aquatic organisms (fish)

Annex Point IIA7.5

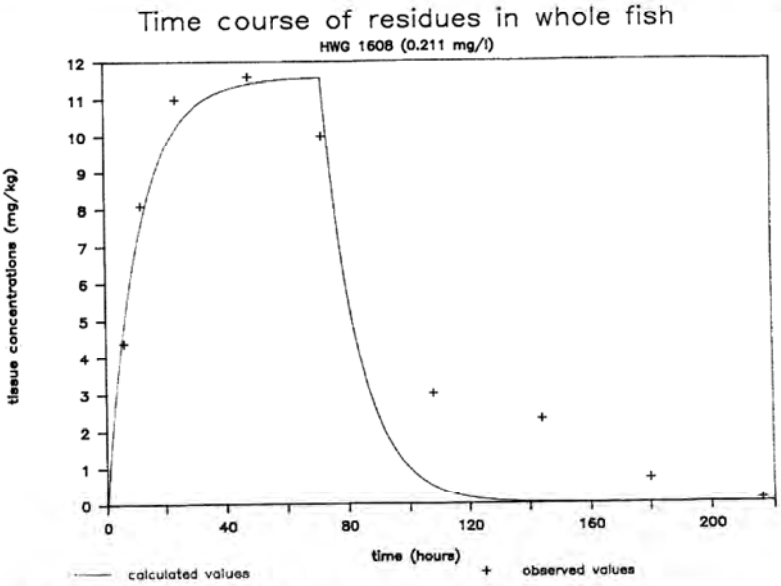
Sample date	Measured concentrations in fish, individual values mg/kg						Mean	±SD
Exposure 0.2 mg/l (nominal)								
hour 0	0.0	0.0	0.0	0.0			0.0	0.0
hour 6	3.9	4.7	4.6	4.6			4.4	0.3
hour 12	7.6	8.4	8.5	7.9			8.1	0.4
hour 24	10.3	10.0	9.9	13.8			11.0	1.9
hour 48	12.2	11.4	12.1	10.7			11.6	0.7
hour 72	11.9	11.2	9.7	9.4	7.9	10.2	10.0	1.4
Depuration								
hour 108	0.4	7.5	1.1				3.0	3.9
hour 144	2.0	1.6	3.5				2.3	1.0
hour 180	0.1	1.5	0.7				0.7	0.7
hour 216	0.0	0.1	0.1	0.5	0.1	0.0	0.1	0.2

Sample date	Measured concentrations in fish, individual values mg/kg						Mean	±SD
Exposure 0.02 mg/l (nominal)								
hour 0	0.00	-0.01	-0.01	0.02			0.00	0.01
hour 6	0.51	0.51	0.52	0.45			0.50	0.03
hour 12	1.08	1.03	0.97	0.72			0.95	0.16
hour 24	1.27	1.27	1.28	1.23			1.26	0.02
hour 48	2.20	2.61	1.37	1.78			1.99	0.54
hour 72	1.48	1.57	1.30	1.32	1.42	2.02	1.52	0.27
Depuration								
hour 108	0.08	0.25	0.03				0.12	0.11
hour 144	0.35	0.19	0.04				0.19	0.16
hour 180	0.03	0.06	0.01				0.04	0.03
hour 216	0.00	0.02	0.04	0.00	0.01	0.02	0.02	0.01



Section A7.4.2 Bioconcentration in aquatic organisms (fish)

Annex Point IIA7.5



4.1.4	Bioconcentration factor (BCF)	Whole fish steady state BCF 93 and 55 for low and high exposure concentration, respectively. Based on total radioactive residues (TRR) and calculated using a non-linear curve fitting program (Duggleby, R. D., 1981, Anal. Biochem. 110, 9-18)
4.1.5	Uptake and depuration rate constants	k_u 5.7 ± 0.3 and 4.8 ± 0.4 1/h for low and high exposure concentration, respectively k_d 0.062 ± 0.005 and 0.088 ± 0.011 1/h for low and high exposure concentration, respectively
4.1.6	Depuration time	DT50 11.2 and 7.9 h for low and high exposure concentration, respectively
4.1.7	Metabolites	Approx. 99% of the radioactivity in the fish at the end of the exposure period was extractable; 63.5% of the radioactivity could be assigned to parent compound, 21.5% to the main metabolite, the glucuronide of a compound (HWG 2061) derived from HWG 1608 by hydroxylation in the tert. butyl moiety; three more, minor metabolites between 1% and 2% were identified, thus yielding a total of 89% of identified radioactivity. Three metabolites with 1.7, 2.5, and 5.7% of the total radioactivity in the fish remained unidentified.
4.1.8	Other Observations	none
4.2	**Estimation of bioconcentration**	The bioconcentration factors at the end of the exposure period of 72 h were determined with 47 ± 7 and 86 ± 15 at the higher and the lower exposure level, respectively. At least in the higher concentration steady state conditions were reached during exposure period. A good accordance of the calculated results with the measured values was reached by fitting the mean uptake values with a non-linear curve fitting programme (8) using the formula: $C_F = (K_1/K_2)*C_W*(1-e^{-K_2*t})$. With this programme, the uptake rate constants were calculated to be 4.8 ± 0.4 and 5.7 ± 0.3 1/h for the high and the low exposure concentrations, respectively. The respective depuration constants came out to be 0.088 ± 0.011 and 0.062 ± 0.005 1/h giving steady state bioconcentration factors

of 55 and 93 for the high and the low exposure concentrations, respectively.

According to the results of this study the compound tested is rapidly taken up by fish and concentrated by a factor of about 55 to 93 when related to total radioactive residues and by a factor of about 35-59 when related to parent compound. When exposure ceases the compound is depurated very rapidly.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

A dynamic 9-day study according to OECD-guideline 305E was conducted to evaluate the bioconcentration of ^{14}C-HWG 1608 by bluegill sunfish (*Lepomis macrochirus*). A microcomputer controlled dosing system maintained mean water concentrations of approximately 0.211 (± 0.003) mg/ml (high level) or 0.018 (± 0.001) mg/l (low level), respectively, of [phenyl-UL-^{14}C] HWG 1608 for a 3-day exposure period. To measure the elimination of HWG 1608-related radioactivity, the test fish were placed in clean water for a 6 day depuration period Fish samples were withdrawn for radioanalysis at intervals during both exposure and depuration period. At the end of the exposure phase additional fishes were removed and extracted; the extracts were analysed for parent compound and (bio) degradation products with chromatographic methods.

5.2 Results and discussion

The bioconcentration factors at the end of the exposure period of 72 h were determined with 47 ± 7 and 86 ± 15 at the higher and the lower exposure level, respectively. At least in the higher concentration steady state conditions were reached during exposure period.

A good accordance of the calculated results with the measured values was reached by fitting the mean uptake values with a non-linear curve fitting program using the formula:

$$C_F = (K_1/K_2)*C_W*(1-e^{-K_2*t}).$$

With this programme, the uptake rate constants were calculated to be 4.8 ± 0.4 and 5.7 ± 0.3 1/h for the high and the low exposure concentrations, respectively. The respective depuration constants came out to be 0.088 ± 0.011 and 0.062 ± 0.005 1/h giving steady state bioconcentration factors of 55 and 93 for the high and the low exposure concentrations, respectively.

According to the results of this study the compound tested is rapidly taken up by fish and concentrated by a factor of about 55 to 93 when related to total radioactive residues and by a factor of about 35-59 when related to parent compound. When exposure ceases the compound is depurated very rapidly.

5.3 Conclusion

Validity criteria of the OECD guideline 305E were fulfilled

5.3.1 Reliability [redacted]

5.3.2 Deficiencies [redacted]

Section A7.4.2 Bioconcentration in aquatic organisms (fish)

Annex Point IIA7.5

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	02 04 04
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	
	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*
Acceptability	
Remarks	

Section A7.4.3.1 Prolonged toxicity to fish

Annex Point IIIA XIII.2.1

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		1 REFERENCE
1.1	**Reference**	[redacted] (1999): HT 308 technical. Fish (rainbow trout), prolonged toxicity test, 21 days (semi-static); [redacted], Report No. FVR60962 (unpublished), 1999-08-26
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes OECD guideline No. 204 (1984)
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]
		3 MATERIALS AND METHODS
3.1	**Test material**	HT 308 technical (tebuconazole)
3.1.1	Lot/Batch number	Batch No.: [redacted]
3.1.2	Specification	As given in section 2 of dossier
3.1.3	Purity	[redacted]
3.1.4	Composition of Product	-
3.1.5	Further relevant properties	Water solubility = 0.032 g/l at 20 °C; Log Pow = 3.7 at 20 °C
3.1.6	Method of analysis	HPLC
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	The 2 top concentrations of 0.32 and 1.0 mg a.i./L were prepared by direct weighing of the test substance without the use of a stock solution. All remaining concentration levels were prepared using a stock solution of 32 mg a.i./L prepared with dilution water. The stock solution was treated with ultrasound (45 min, 40 °C).
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	-

Section A7.4.3.1 Prolonged toxicity to fish

Annex Point IIIA XIII.2.1

3.4	**Testing procedure**	
3.4.1	Dilution water	see table A7_4_3_1-1
3.4.2	Test organisms	*Oncorhynchus mykiss*, see table A7_4_3_1-2
3.4.3	Test system	Semi-static, see table A7_4_3_1-3
3.4.4	Test conditions	see table A7_4_3_1-4
3.4.5	Duration of the test	21 days
3.4.6	Test parameter	Mortality and behaviour
3.4.7	Sampling	Observations were performed daily. Temperature, pH-value and oxygen saturation were measured in all vessels at the beginning of the test and on every water exchange (old and new media). Measurement of fish size: A representative sample (10 fish) of the test population has been weighed and measured before the test starts. These fish were not used in the test. All survivors were weighed and measured at the termination of the test.
3.4.8	Monitoring of TS concentration	The test substance was measured at all dosage levels three times out of the freshly prepared media and out of the corresponding 72 h old media.
3.4.9	Statistics	Statistical significance of the achieved data concerning length and weight of the surviving test fish was controlled by a One Way Analysis of Variance using PC program SigmaStat (Windows) rel. 2.00 of Jandel Scientific.
		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: 0.01, 0.032, 0.01, 0.32 and 1.0 mg a.s./L
4.2.2	Actual concentrations of test substance	Actual concentrations of tebuconazole are provided in the report. The mean measured concentrations of test substance ranged from 82 to 111 % of nominal concentrations during the test period for all test levels.

Section A7.4.3.1 Prolonged toxicity to fish

Annex Point IIIA XIII.2.1

4.2.3	Effect data (Mortality)	No dead fish were observed during the study. The no-observed-effect concentration (NOEC) was 0.010 mg a.i./L, the lowest-observed-effect concentration (LOEC) was 0.032 mg a.i./L and the lowest lethal concentration (LLC) was > 1.0 mg a.i./L.
4.2.4	Concentration / response curve	No dead fish were observed during the study.
4.2.5	Other effects	Disturbance of feeding habit was observed in the concentrations from 0.032 to 1.0 mg a.s./L, starting on day 12. A statistically significant difference in body weight between the control and the concentration 0.032 mg a.s./L was observed at the end of the study. At the end of the study body weight and body length were statistically significantly reduced at the concentration from 0.1 to 1.0 mg a.s./L.
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	No dead fish were observed during the study.
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 HT 308 (tebuconazole) to rainbow trout (*Oncorhynchus mykiss*) with prolonged exposure was investigated in a 21-day semi-static experiment in accordance with the OECD guideline No. 204. The test shows no significant deviations from the guideline.
5.2	**Results and discussion**	No dead fish were observed during the study. The no-observed-effect concentration (NOEC) was 0.010 mg a.i./L, the lowest-observed-effect concentration (LOEC) was 0.032 mg a.i./L and the lowest lethal concentration (LLC) was > 1.0 mg a.i./L. Disturbance of feeding habit was observed in the concentrations from 0.032 to 1.0 mg a.s./L, starting on day 12. The NOEC is based on a statistically significant difference in body weight between the control and the concentration 0.032 mg a.s./L at the end of the study. At the end of the study body weight and body length were statistically significantly reduced at the concentration from 0.1 to 1.0 mg a.s./L. The mean measured concentrations of test substance ranged from 82 to 111 % of nominal concentrations during the test period for all test levels. Therefore results are based on nominal test substance concentrations.
5.2.1	21d-LC_{50}	-

Section A7.4.3.1 Prolonged toxicity to fish

Annex Point IIIA XIII.2.1

5.2.2	NOEC	0.010 mg a.i./L
5.2.3	LOEC	0.032 mg a.i./L
5.3	**Conclusion**	The validity criteria are summarised in table A7_4_3_1-5. The no-observed-effect concentration established in this study was 0.010 mg a.s./L.
5.3.1	Other conclusions	-
5.3.2	Reliability	[redacted]
5.3.2	Deficiencies	[redacted]

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	June 2004
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Section A7.4.3.1 Prolonged toxicity to fish

Annex Point IIIA XIII.2.1

[redacted]

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

Table A7_4_3_1-1: Dilution water

Criteria	Details
Source	Tap water with local origin. The water was filtered on activated charcoal and aerated for at least 24 hours. The water is analysed according to the German tap water prescription.
Alkalinity	-
Hardness	62 mg of $CaCO_3$/L
pH	7.31 (Control at day 0 of the test)
Oxygen content	98 % of the air saturation value (Control at day 0 of the test)
Conductance	-
Holding water different from dilution water	No

Table A7_4_3_1-2: Test organisms

Criteria	Details
Species/strain	Rainbow trout (*Oncorhynchus mykiss*)
Source	[redacted]
Wild caught	No
Age/size	Mean body length 5.5 cm, mean body weight 1.8 g
Kind of food	"Kronen Fish Allround 2 mm" from Ssniff Spezialdiäten GmbH
Amount of food	Fish were fed with 2% of their initial body weight for days 1-10, and with 3% of their initial body weight for days 11-21
Feeding frequency	Fish were fed daily
Pretreatment	12 days of acclimatisation
Feeding of animals during test	Yes

Table A7_4_3_1-3: Test system

Criteria	Details
Test type	Semi-static test conditions
Renewal of test solution	Test medium renewal was performed three times a week
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_1-4: Test conditions

Criteria	Details
Test temperature	15 ± 2 °C
Dissolved oxygen	> 60% of the air saturation value
pH	7.08-7.60
Adjustment of pH	No
Aeration of dilution water	Yes Continuous aeration was provided
Intensity of irradiation	0.1-1 µmol/m^2 s
Photoperiod	12 hours photoperiod daily

Table A7_4_3_1-5: 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 ≥ 80% of initial concentration during test	X	

Criteria for poorly soluble test substances	-	-

Section 7.4.3.2 Effects on reproduction and growth rate of fish
Annex Point IIIA XIII 2.2

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		1 REFERENCE
1.1	**Reference**	[redacted], 1988, The toxicity of HWG 1608 technical to rainbow trout (salmo gairdneri) embryos and larvae. [redacted], PF-Report No. 96723 (+ 99628 as addendum) SLS Report No. #87-11-2545, April 1, 1988
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes / EPA Reference No. 72-4, also reference to internal protocols and methods for egg fertilization and handling by from literature (Leitritz, 1959, Trout and Culture. State of California Dept. of Fish and Game, Fish Bulletin No. 164, 196 pp)
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]
		3 MATERIALS AND METHODS
3.1	**Test material**	HWG 1608 (tebuconazole) technical grade
3.1.1	Lot/Batch number	Lot number #[redacted]
3.1.2	Specification	Technical grade
3.1.3	Purity	[redacted]% of active substance
3.1.4	Composition of Product	n.a.
3.1.5	Further relevant properties	Stable under test conditions, not volatile, water solubility: 32 mg/l
3.1.6	Method of analysis	HPLC reversed phase
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	A stock solution containing 2.5 mg Tebuconazole / ml acetone was diluted in a flow through system
3.3	**Reference substance**	Acetone was used for the control of dilution
3.3.1	Method of analysis for reference substance	HPLC, reversed phase

Section 7.4.3.2 Effects on reproduction and growth rate of fish
Annex Point IIIA XIII 2.2

3.4	**Testing procedure**	
3.4.1	Dilution water	see table A7_4_3_2-2
3.4.2	Test organisms	see table A7_4_3_2-3
3.4.3	Handling of embryos and larvae (OECD 210/212)	After fertilization the eggs were allowed to be 45 minutes undisturbed then the eggs were incubated in egg cups 50 eggs each / two cups per aquarium. After three hours at maximum the a.i. was applied to the water. Egg fertilisation was evaluated after day 15. Then dead eggs were removed. To determine the swim-up phase in each aquarium 20 viable eggs were incubated in egg cups until no more than 5 unhatched viable embryos remained in the control. This occurred at day 23. In the end 20 larvae in each aquarium were used for the 60 days post –hatch larvae exposure.
3.4.4	Test system	see table A7_4_3_2-4
3.4.5	Test conditions	see table A7_4_3_2-5
3.4.6	Duration of the test	83 days (60 days after hatching)
3.4.7	Test parameter	
3.4.8	Sampling	Day 0: fertilisation of the eggs, day 15: fertilisation (viability) control, day 23: hatching control and begin of the 60 days larvae exposure. In addition to samplings and measurements, observations on the behaviour of the larvae were made (e.g. at day 40) made but not detailed reported.
3.4.9	Monitoring of TS concentration	Yes, every week
3.4.10	Statistics	
	4 RESULTS	
4.1	**Range finding test**	
4.1.1	Concentration	0.16 – 2.5 mg a.i. /l
4.1.2	Number/ percentage of animals showing adverse effects	Nearly no larvae survival at 1.3 and 2.5 mg/l after 48 days (20 days post hatch) 9%, 24 % and 64 % of the larvae at concentrations of 0.63, 0.31 and 0.16 mg/l tebuconazole has successfully developed to the swim up stage.
4.1.3	Nature of adverse effects	larvae mortality and delayed larvae development, e.g. reduced length and wet weights
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	15, 30, 60, 120 and 240 µg/l tebuconazole
4.2.2	Actual concentrations of test substance	see table A7_4_3_2-6

Section 7.4.3.2 Effects on reproduction and growth rate of fish
Annex Point IIIA XIII 2.2

4.2.3	Effect data	see table A7_4_3_2-7 From table it can be derived that the larvae at the concentrations above 12 µg/l tebuconazole /l show significant lower survival rates, lengths and weights. For other observed effects see under 1.1.3 fertility and hatching was not affected by all applied concentrations. From the experiment a NOEC of 12 µg tebuconazole /l and a LOEC of 25 µg/l is derived-
4.2.4	Concentration / response curve	Only given in the report
4.2.5	Other effects	At the tested concentration above 12 µg/l larvae were dark coloured and show a retardant behaviour like lethargy and loss of equilibrium.
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	No adverse effects were visible
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 methods used to perform the study are similar to the OECD Guideline 210 issued 1992. Some deviations like a slightly higher temperature and a drop of the oxygen content in some samples are already addressed by the authors. They are considered as not relevant, taking into account the results of the solvent control and control experiments.
5.2	**Results and discussion**	In the tested range of concentrations of tebuconazole from 12 – 230 µg/l (measured concentrations) no adverse effects were seen concerning the fertilization and the hatching of the eggs. Concerning the development of the larvae only at the lowest concentration (12 µg/l) no significant effects were observed. At 250,120 and 61 µg/l survival of the larvae was strongly diminished. At 25 µg/l survival increased, but there were still developmental effects concerning total lengths and weights. In addition the behaviour of the larvae was affected showing e.g. lethargy and loss of equilibrium.
5.2.1	NOEC	0.012 mg/l (12 µg/l)
5.2.2	LOEC	0.025 mg/l (25 µg/l)
5.3	**Conclusion**	With slight deviation modifications validity criteria can be considered as fulfilled. A dose response relationship is established for the endpoints on larvae survival and development.

Section 7.4.3.2 Effects on reproduction and growth rate of fish
Annex Point IIIA XIII 2.2

The validity criteria are summarised in table A7_4_3_2-8.

5.3.1 Other conclusions

5.3.2 Reliability [redacted]

5.3.2 Deficiencies [redacted]

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*02 04 04*
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	
	COMMENTS FROM ... *(specify)*
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_3_2-2: Dilution water

Criteria	Details
Source	Well water
Alkalinity ($CaCO_3$)	27-33 mg/l
Hardness ($CaCO_3$)	27-33 mg/l
pH	7.0-7.1
Oxygen content	7.5 – 8.8 mg Oxygen / l
Conductance	100-130 µmho / cm
Holding water different from dilution water	No

Table A7_4_3_2-3: Test organisms

Criteria	Details
Species	Unfertilized eggs and sperm from rainbow trout
Source	[redacted]
Wild caught	No
Age/size	
Kind of food	*Artemia salina*
Amount of food	*Ad libitum*
Feeding frequency	3-2 times daily
Post-hatch larvae exposure	60 days
Time to first feeding	about 13 days post hatch
Feeding of animals during test	yes
Treatment for disease within 2 weeks proceeding test	no

Table A7_4_3_2-4: Test system

Criteria	Details
Test type	Flow-through
Renewal of test solution	During 24 hours a 6.5 fold vessel volume is provided, which cause during 8 hours a 90 % replacement of the test water
Volume of test vessels	15 l aquaria
Volume/animal	n.a (growing animals)
Number of animals/vessel	2 x 50 eggs in egg cups 20 / larvae during the 60 days post- hatch exposure
Number of vessels/ concentration	two for each concentration, control and acetone control
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_3_2-5: Test conditions

Criteria	Details
Test temperature	14 °C ± 0.8 °C (11-15 °C from measurements of the water bath)
Dissolved oxygen	7.5 – 8.8 mg Oxygen / l
pH	6.5 –7.9
Adjustment of pH	No
Aeration of dilution water	Yes, pre-treatment
Intensity of irradiation	320 – 720 lux per m²(30-70 footcandle) at water surface
Photoperiod	16 h photoperiod daily

Table A7_4_3_2-6 **Results of the analysis of the exposure solution for tebuconazole during the early life stage exposure of rainbow trout**

Nominal concentration µg/l	Mean measured concentration (S.D.)[a] µg/l	Percent of nominal concentration	Number of samples
240	230 (± 35)	96	24
120	120 (± 16)	100	24
60	61 (± 11)	102	24
30	25 (± 4.9)	83	24
15	12 (± 1.7)	80	23[b]
Solvent control	< 3.4		24
Control	< 3.4		24

a) mean until day 77, data from day 83 were regarded as invalid (integrity of the samples was not maintained),
b) one sample was below the detection limit and was regarded as outliner

Table A7_4_3_2-7 **Embryo viability, survival of organisms at hatch and survival, total length and wet weight of rainbow trout larvae exposed to tebuconazole for 83 days (60 days post-hatch).**

Measured Concentration[2] (µg/l)	Embryo Viability (%)	Survival of Organisms at Hatch (%)	Larvae (60 days post-hatch)		
			Larvae survival (%)	Mean total length (mm)	Mean wet weight (g)[1]
230 A	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
230 B	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
230 mean	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
120 A	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
120 B	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
120 mean	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
61 A	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
61 B	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
61 mean	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
25 A	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
25 B	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
25 mean	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
12 A	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

Measured Concentration[2] (µg/l)	Embryo Viability (%)	Survival of Organisms at Hatch (%)	Larvae (60 days post-hatch) Larvae survival (%)	Mean total length (mm)	Mean wet weight (g)[1]
12 B	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
15 mean	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Solv. cont. A	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Solv. cont. B	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Solv. cont. mean	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Control A	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Control B	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Control mean	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Pooled Controls	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

1) Values presented in parentheses equal standard deviation

2) Values on larvae survival (and development) from the 230, 120, 61 and 25 µg tebuconazole/l samplings are statistical ($P \leq 0.05$) and biological significant different from the pooled control data

Table A7_4_3_2-8: Validity criteria for fish tests according to OECD Guidelines 210/212

	Fulfilled	Not fulfilled
Concentration of dissolved oxygen > 60% saturation throughout the test	**yes**[1]	
Difference of water temperature < 1.5% between test chambers or successive days at any time during test; temperature within range for specific test species	**yes**[2]	
Overall survival of fertilized eggs in controls (and solvent controls) ≥ value, specified for the specific test species	**yes**	
Test substance concentrations maintained within ± 20% of mean measured values	**yes**	
No effect on survival nor any other adverse effect found in solvent control	**yes**	
Further criteria for poorly soluble test substances	**n.a.**	

1 and 2: slight deviations discussed in the report and regarded to be not relevant

Section 7.4.3.3.1 Annex Point IIIA 12.2	Bioaccumulation in an appropriate invertebrate species	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [...]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ].**	
Detailed justification:	No study on submitted on bioaccumulation on an invertebrate species. Because the water solubility of tebuconazole is relatively high and the measured bioconcentration in fish is below 100, there is no indication for further testing on bioaccumulation in aquatic invertebrates.	
Undertaking of intended data submission [ ]	–	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	Date of action: 13. September 2004	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

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

Official use only

		1 REFERENCE
1.1	**Reference**	Noack M (1999): HT 308 technical - *Daphnia magna* reproduction test (21 d), Dr. U. Noack-Laboratorium, Sarstedt, Germany, Report No. DRE60961 (unpublished), 1999-08-23
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes OECD guideline No. 211 (1997)
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]
		3 METHODS
3.1	**Test material**	HT 308 technical (tebuconazole)
3.1.1	Lot/Batch number	Batch no.: [redacted]
3.1.2	Specification	As given in section 2 of dossier
3.1.3	Purity	[redacted]
3.1.4	Composition of Product	-
3.1.5	Further relevant properties	Water solubility: 0.032 g/l at 20 °C; Vapour pressure: negligible; Log Pow = 3.7 at 20 °C
3.1.6	Method of analysis	HPLC
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	The test media were prepared using a stock solution of 10 mg a.i./L. The stock solution was treated with ultrasound (45 min, 40 °C).
3.3	**Reference substance**	Yes, Potassium dichromate
3.3.1	Method of analysis for reference substance	-

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

3.4	**Testing procedure**	
3.4.1	Dilution water	See table A7_4_3_4-1
3.4.2	Test organisms	*Daphnia magna*. See table A7_4_3_4-2
3.4.3	Handling of offspring	Reproduction success was measured by controlling the number of juveniles per parent.
3.4.4	Test system	see table A7_4_3_4-3
3.4.5	Test conditions	see table A7_4_3_4-4
3.4.6	Duration of the test	21 days
3.4.7	Test parameter	Number of living offspring per parent animal
3.4.8	Examination / Sampling	Mortality of parent Daphnia was observed and recorded once a day. First appearance of juveniles was checked daily. Abnormalities were checked and recorded at the observation dates. Dissolved oxygen concentration, water temperature and pH-value were measured once a week, in old and fresh media, in one replicate of the control and highest test substance concentration. At the end of the test the total length of each Daphnia and body weight of live Daphnia were determined.
3.4.9	Monitoring of TS concentration	Yes, The concentrations of the active ingredient were determined in the old and new media once per week in the concentration range 0.01-0.9 mg a.s./L and the control.
3.4.10	Statistics	The NOEC and LOEC were determined directly from the test results obtained. Statistical significance of the achieved data (number of juveniles and length of the parent animals) were controlled by Kruskal-Wallis one way analysis of variance on ranks and multiple comparison method of Dunett's with the software SigmaStat of Jandel Scientific version no. 2.0 (Windows).
		4 RESULTS
4.1	**Range finding 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: 0.01, 0.03, 0.1, 0.3 and 0.9 mg a.s./L

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

4.2.2	Actual concentrations of test substance	Actual concentrations of the test substance are provided in the report. Mean values of recovery rates including new and old media were in the range of 87 and 136 %.
4.2.3	Effect data	No significant mortality and immobilisation of parental occurred after 21 days. The average number of juveniles per parent in the control group was 70 after 21 days. At concentrations 0.03-0.9 mg a.s./L a statistical significant difference was noted on the reproduction output vs. control (43-95%). The coefficient of variation around the mean number of living offspring per parent in the control group was < 25%. The EC_{50} for reproduction was calculated to be 0.14 mg/L. The no-observed-effect concentration (NOEC) was 0.01 mg a.i./L and the lowest-observed-effect concentration (LOEC) was 0.03 mg a.i./L. Significant numbers of stillborn and eggs without hatching of juveniles were observed in the highest test concentration of 0.9 mg a.s./L.
4.2.4	Concentration / response curve	Provided in the report
4.2.5	Other effects	Body weight and body length was not affected at all test concentrations.
4.3	**Results of controls**	No significant mortality and immobilisation of parental occurred after 21 days.
4.4	**Test with reference substance**	Performed
4.4.1	Concentrations	0.58, 1.0, 1.8, 3.2 and 5.8 mg a.s./L
4.4.2	Results	EC_{50} (24 h) = 1.9 mg/L

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The effects of HT 308 technical (tebuconazole) on reproduction capacity of *Daphnia magna* and other substance related effects on parameters such as body weight, immobilisation of the parentals, time of production of first brood, aborted eggs and stillborn juveniles were investigated in a semi – static test which prolonged to 21 days. Test organisms were exposed to aqueous test medium containing the test substance at various concentrations. The study follows OECD guideline No. 211 (1997) and shows no significant deviations.

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

5.2	**Results and discussion**	In the control group and in all tested concentrations no significant mortality and immobilisation of parental occurred after 21 days. The average number of juveniles per parent in the control group was 70 after 21 days. At concentrations 0.03-0.9 mg a.s./L a statistical significant difference was noted on the reproduction output vs. control (43-95%). The coefficient of variation around the mean number of living offspring per parent in the control group was < 25%. The EC_{50} for reproduction was calculated to be 0.14 mg/L. The no-observed-effect concentration (NOEC) was 0.01 mg a.i./L and the lowest-observed-effect concentration (LOEC) was 0.03 mg a.i./L. All reported results are based on nominal concentrations. Significant numbers of stillborn and eggs without hatching of juveniles were observed in the highest test concentration of 0.9 mg a.s./L. Body weight and body length was not affected at all test concentrations. Mean values of recovery rates including new and old media were in the range of 87 and 136 %.
5.2.1	NOEC	0.01 mg a.s./L
5.2.2	LOEC	0.03 mg a.s./L
5.2.3	EC_{50} (EC_x)	0.14 mg a.s./L
5.3	**Conclusion**	Validity criteria are given in table A7_4_3_4-5. The NOEC (21 d) for the reproduction of water fleas was determined to be 0.01 mg a.s./L.
5.3.2	Reliability	[redacted]
5.3.2	Deficiencies	[redacted]

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

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	June 2004
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

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

[redacted]

	COMMENTS FROM ... *(specify)*
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	
Remarks	

Table A7_4_3_4-1: Dilution water

Criteria	Details
Source	Culture medium: B.P. ELENDT M4, according to selenium deficiency in Crustaceae, Protoplasma 154, 25-33 (1990) modified to a total hardness of 160-180 mg CaCO3/L
Salinity	-
Hardness	168 mg CaCO3/L (initial value of control)
pH	7.89 (initial pH of control)
Ca / Mg ratio	-
Na / K ratio	-
Oxygen content	8.7 mg/L (initial value of control)
Conductance	-
TOC	-
Holding water different from dilution water	No

Table A7_4_3_4-2: Test organisms

Criteria	Details
Strain / Clone	*Daphnia magna* STRAUS (clone 5)
Source	Institut für Wasser-, Boden- und Lufthygiene des Bundesgesundheitsamtes, D-14195 Berlin
Age	2-24 hours
Breeding method	Daphnids were bred in test facility in 2-3 L glass vessels with approximately 1.8 L culture medium at 21 °C in an incubator; 16 h illumination (1.5-3 µmol/m^2 s (100-200 lx)). Culture medium: B.P. ELENDT M4, according to selenium deficiency in Crustaceae, Protoplasma 154, 25-33 (1990) modified to a total hardness of 160-180 mg $CaCO_3$/L
Kind of food	*Scenedesmus subspicatus*
Amount of food	0.4-1.4 ml suspension of algae per test vessel (0.1-0.2 mg C per Daphnia and day)
Feeding frequency	Every day
Pretreatment	-
Feeding of animals during test	Yes see above

Table A7_4_3_4-3: Test system

Criteria	Details
Test type	Semi - static
Renewal of test solution	Renewal of test solution three times per week
Volume of test vessels	50 ml
Volume/animal	50 ml
Number of animals/vessel	1
Number of vessels/concentration	10
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_3_4-4: Test conditions

Criteria	Details
Test temperature	18-22 °C ± 2 °C
Dissolved oxygen	Final oxygen concentration was ≥ 8 mg/L
pH	7.89 – 8.74 (initial pH for control), 7.22 – 8.11 (final pH for control); 7.64 – 8.19 (initial pH for highest test substance concentration), 7.24 – 8.17 (final pH for highest test substance concentration)
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	Max. 800 lux
Photoperiod	16 hours

Table A7_4_3_4-5: 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	

Criteria for poorly soluble test substances	-	-

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

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		1 REFERENCE
1.1	**Reference**	Burgess D., 1988, Chronic Toxicity of HVG-1608 to *Daphnia magna* Under flow-Through Test Conditions, Analytical Bio-Chemistry Laboratories, Inc, report number 96792, June 10, 1988.
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes ASTM and U.S. EPA
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]
		3 METHODS
3.1	**Test material**	As given in section 2 HWG-1608
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2
3.1.3	Purity	analytical purity in % of active substance = [redacted]
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_3_4-1

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

3.3	**Reference substance**	Yes Acetone
3.3.1	Method of analysis for reference substance	No
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	Reproduction success was measured by counting and discarding the offspring produced in each concentration every Monday, Wednesday, and Friday for the duration of the study.
3.4.4	Test system	see table A7_4_3_4-4
3.4.5	Test conditions	see table A7_4_3_4-5 Five concentration plus control and solvent control. The mean measured concentrations, as determined by HPLC were 0.042, 0.069, 0.12, 0.23, and 0.51 mg/l.
3.4.6	Duration of the test	21-days
3.4.7	Test parameter	Daphnid survival, the mean young/adult/reproduction, and the adult daphnid mean length.
3.4.8	Examination / Sampling	Survival, abnormal effects and observance of first brood of the organisms were recorded daily. Reproduction success was measured by counting and discarding the offspring produced in each concentration every Monday, Wednesday, and Friday. Upon termination of the test on day 21 of exposure, the length of each adult daphnid was measured.
3.4.7	Monitoring of TS concentration	Yes Exposure concentrations of HWG-1608 were measured by HPLC on days 0, 4, 7, 14, and 21
3.4.8	Statistics	The measured parameters of survival, adult length, and total young/adult/reproduction were analysed using a one-way analysis of variance (ANOVA). When treatment effects were indicated following a significant F-test of the mean square ratios, a multiple means comparison test, Dunnett´s was used to determine which exposure levels differed from the control values. All differences were considered significant at the $\alpha = \leq 0.05$ level (95% confidence level).
		4 RESULTS
4.1	**Range finding test**	Performed
4.1.1	Concentration	A 7 – day study at 0.18, 0.36, 0.75, 1.5, and 3.0 mg/l
4.1.2	Number/ percentage of animals showing adverse effects	After 7 days of exposure, effects were observed in all levels

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

4.1.3 Nature of adverse effects

4.2 Results test substance

4.2.1 Initial concentrations of test substance

Measured initial concentrations of test substance see point 4.2.2. The mean measured HWG-1608 concentrations during the experiment was 0.042, 0.069, 0.12, 0.23, and 0.51 mg/l

4.2.2 Actual concentrations of test substance

Measured Concentrations in mg/l

	Day 0	Day 4	Day 7	Day 14	Day 21	Mean
Control	<0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010*
Solvent Control	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010
Level 1	0.023	0.034	0.041	0.044	0.074	0.042
Level 2	0.047	0.057	0.072	0.075	0.11	0.069
Level 3	0.081	0.10	0.13	0.13	0.21	0.12
Level 4	0.17	0.17	0.24	0.25	0.41	0.23
Level 5	0.32	0.40	0.50	0.57	0.79	0.51
*Detection limit was 0.010 mg/l						

4.2.3 Effect data

The total numbers of living offspring per parent animal alive at test termination

control	Solvent control	0.042 mg/l	0.069 mg/l	0.12 mg/l	0.23 mg/l	0.51 mg/l
80	80	79	80	84	55	23

The number of dead parent animals (including time of death)

control	Solvent control	0.042 mg/l	0.069 mg/l	0.12 mg/l	0.23 mg/l	0.51 mg/l
0	**0**	**0**	**1** 1 day19	**0**	**1** 1 day13	**10** 1 day 11 3 day 15 5 day 16 1 day 20

After a 21-day exposure to HWG-1608 daphnid survival was significantly different ($P < 0.05$) from the controls in 0.51 mg/l. A day 21 EC_{50} was calculated to be 0.33 mg/l

For reproduction NOEC was 0.12 mg/l and LOEC was 0.23 mg/l.

All results were based on the mean measured concentrations of HWG-1608

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

4.2.4	Concentration / response curve	
4.2.5	Other effects	The daphnid lengths in the HWG-1608 mean measured concentrations of 0.23 and 0.51 mg/l were significantly different from the controls.
4.3	**Results of controls**	See above
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**	Follow OECD Guideline no. 211. No important derivations from this guidelines
5.2	**Results and discussion**	Based on the statistical analysis of survival, young/adult/reproduction day, and adult mean length, from this 21-day *Daphnia magna* dynamic life cycle study, the MATC (Maximum Acceptable Toxicant Concentration) limits were estimated to be the mean measured concentration of > 0.12 and < 0.23 mg/l.
5.2.1	NOEC	0.12 mg/l
5.2.2	LOEC	0.23 mg/l
5.2.3	EC_{50} (EC_x)	After a 21-day exposure to HWG-1608 daphnid survival was significantly different ($P < 0.05$) from the controls in 0.51 mg/l. A day 21 EC_{50} was calculated to be 0.33 mg/l
5.3	**Conclusion**	There is a dose response relationship in concentrations above 0.12 mg/l. Validity criteria can be considered fulfilled.
5.3.2	Reliability	[redacted]
5.3.2	Deficiencies	[redacted]

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

Evaluation by Competent Authorities	
Date	**EVALUATION BY RAPPORTEUR MEMBER STATE**
Materials and Methods	*02 04 04*
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	[redacted]
Date	**COMMENTS FROM ...** *(specify)*
Materials and Methods	*Give date of comments submitted*
Results and discussion	*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*
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	*Discuss if deviating from view of rapporteur member state*

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

Criteria	Details
Dispersion	No
Vehicle	Yes acetone
Concentration of vehicle	? (% v/v)
Vehicle control performed	Yes
Other procedures	

Table A7_4_3_4-2: Dilution water

Criteria	Details
Source	ABC well water
Salinity	?
Hardness	206 – 275 ppm
PH	7.6 – 8.4
Ca / Mg ratio	?
Na / K ratio	?
Oxygen content	7.4 – 9.1 ppm (dissolved oxygen
Conductance	500 – 650 µmhos/cm
TOC	1.4 ppm (0.9 – 2.8 ppm)
Holding water different from dilution water	Yes

Table A7_4_3_4-3: Test organisms

Criteria	Details
Strain / Clone	*Daphnia magna*
Source	In house *daphnid* culture
Age	First-instar daphnids < 24 hours old
Breeding method	
Kind of food	Suspension of algae (*Selenastrum capricornutum*) supplemented with a Tetramin/cereal leaves/yeast suspension.
Amount of food	Fed with an algal suspension three times daily providing approximately 2-4 x 10^8 cells to each test unit on each day. Daphnids were supplemented once daily with 0.2 ml per test chamber of a 9.0 mg/ml suspension of Tetramin, cereal leaves, vitamins and yeast given a final suspended solids concentration of 1.8 mg/l.
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	Flow-through
Renewal of test solution	Aerated test solution was delivered to each test chamber at an average rate of 4.0 ml/min, an amount which was sufficient to replace the 1-liter test volume at least 5.8 times in a 24-hour period.
Volume of test vessels	1000 ml
Volume/animal	100 ml
Number of animals/vessel	10
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 (measured daily)
Dissolved oxygen	Ranged from 8.1 to 9.0 mg/l, represented 93 and 103% saturation at 20 °C. Measured days 0, 4, 7, 14 and 21
pH	8.0 – 8.2 (measured day 0, 4, 7, 14 and 21)
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	The lighting was 40-80 foot-candles
Photoperiod	16-hour daylight and a 8- hour darkness

Table A7_4_3_4-6: 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	

Criteria for poorly soluble test substances	X	

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA, XIII.3.4 *Chironomus riparius*

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1 REFERENCE

1.1	**Reference**	Dogerloh, M. (2003): Influence of tebuconazole (tech.) on development and emergence of larvae of *Chironomus riparius* in a water sediment system, Bayer CropScience AG, Germany, Report No. DOM 22066 (unpublished), February 6, 2003
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	BBA-proposal: "Effects of plant protection products on the development of sediment-dwelling larvae of *Chironomus riparius* in a water-sediment system" (1995). Proposal for a new OECD Guideline 219: "Sediment-Water Chironomid Toxicity Test using Spiked Water" (February 2001)
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 METHODS

3.1	**Test material**	Tebuconazole (tech.)
3.1.1	Lot/Batch number	Batch No. [redacted]
3.1.2	Specification	AS given in section 2 of dossier
3.1.3	Purity	[redacted]
3.1.4	Composition of Product	-
3.1.5	Further relevant properties	-
3.1.6	Method of analysis	For chemical analysis of the active ingredient additional parallel replicates were prepared for analytical purposes only (control: one replicate; 1.0, 3.2 and 10 mg/l: two replicates). Three times during the study (1 hour, 7 and 28 days after application) one test container of each nominal initial test substance concentrations of 1.0, 3.2 and 10 mg/L was removed from the study (1 hour and seven days after application the parallel replicates were taken). The overlying water of these test containers was carefully decanted. The sediment of each beaker was filtered by vacuum. This filtrate (= pore water) and the overlying water were analysed by means of HPLC.

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA, XIII.3.4 *Chironomus riparius*

3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	0.0786 g test substance were given to 2000 ml M7 medium to obtain the stock solution (38 mg a.s./L). The stock solution was stirred on a magnetic stirrer for 10 minutes and treated in an ultrasonic bath for 10 minutes. In order to establish the concentrations of 1.0, 1.8, 3.2, 5.6 and 10.0 mg a.s./L appropriate volumes of the stock solution were applied into the overlying column of the beakers. Aliquots of the stock solutions were applied just below the water surface with a pipette. Gentle mixing of the water ensured homogeneous distribution without disturbance of the sediment.
3.3	**Reference substance**	Not performed
3.3.1	Method of analysis for reference substance	-
3.4	**Testing procedure**	
3.4.1	Dilution water Test Sediment	Details on Dilution water see table A7_4_1_2-1. The test sediment used was artificial sediment which was prepared 7 days before the start of the test. It consists of 74% fine quartz sand, 5% dried, finely ground peat, 20% kaolin and around 1% calcium carbonate. Details on test sediment see table A7_4_1_2-1a
3.4.2	Test organisms	*Chironomus riparius*, see table A7_4_1_2-2
3.4.3	Test system	see table A7_4_1_2-3
3.4.4	Test conditions	see table A7_4_1_2-4
3.4.5	Duration of the test	28 days
3.4.6	Test parameter	The sex, time of emergence and number of emerged midges
3.4.7	Sampling	The test vessels were observed at least three times per week to make a visual assessment of any behavioural differences compared to the control. The sex, time and number of emerged or not fully emerged adults were recorded daily during the period of emergence. The pH-value, the dissolved oxygen content and the temperature were measured once per week.
3.4.8	Monitoring of TS concentration	Yes, the concentration of tebuconazole was analysed in the overlying water and the pore water of the sediment. Three times during the study (1 hour, 7 and 28 days after application) one test container of each nominal initial test substance concentrations of 1.0, 3.2 and 10 mg/L was removed from the study (1 hour and seven days after application the parallel replicates were taken). In addition the overlying water and the pore water of the control were also analysed on day 0.
3.4.9	Statistics	Statistical analysis was performed by employing a computerised program. χ^2-test was performed to establish different sensitivities of sexes and probit analysis was performed to calculate the EC_{15} and EC_{50} for numbers of emerged midges.

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA, XIII.3.4 *Chironomus riparius*

		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, 1.8, 3.2, 5.6 and 10.0 mg a.s./L
4.2.2	Actual concentrations of test substance	Actual concentrations: 0.729, 1.31, 2.33, 4.08 and 7.29 mg a.s./L
4.2.3	Effect data	Number of emerged midges see table A7_4_1_2-5. The influence of tebuconazole on the emergence and development of *Chironomus riparius* after 28 days (EC_5, EC_{10}, EC_{15} and EC_{50}) is shown in table A7_4_1_2-6.
4.2.4	Concentration / response curve	Dose-effect-curve on number of emerged midges (sum of male and female midges) is given in the report.
4.2.5	Other effects	Increasing test substance concentrations inhibit the mature of the larvae.
4.3	**Results of controls**	95% of the inserted larvae maturated to adults in the control
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**	To assess the influence of tebuconazole (tech.) on development and emergence of larvae of *Chironomus riparius* in a water-sediment system a study was performed according to the BBA-proposal: "Effects of plant protection products on the development of sediment-dwelling larvae of *Chironomus riparius* in a water-sediment system" (1995). Proposal for a new OECD Guideline 219: "Sediment-Water Chironomid Toxicity Test using Spiked Water" (February 2001) In a 28-day static test system larvae of *Chironomus riparius* were exposed to different concentrations of the test substance in a water/sediment system. The test shows no significant deviations from the BBA-Guideline.

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA, XIII.3.4 *Chironomus riparius*

5.2	**Results and discussion**	The influence of tebuconazole on the emergence and development of *Chironomus riparius* after 28 days (EC_5, EC_{10}, EC_{15} and EC_{50}, probit analysis) is shown in table A7_4_1_2-6. The EC_{15} (28 d) for *Chironomus riparius* was determined to be 2.51 mg a.s./L. Because the measured amount of a.s. of the application solution on day 0 was only 72.9 %, all statistical calculations were related to initial measured concentrations. The start of emergence was not delayed at any test concentrations where emergence occurred. However, no emergence at all was observed at test concentrations of 4.08 and 7.29 mg a.s./L. The EC_{15} (probit analysis) for the development rate of male, female and pooled sex could not be calculated, as the influence on the development rate was 15 % at less than all test concentrations where emergence rates were comparable to those in the control. The summary of numbers of emerged midges over 28 days is given in Table A7_4_1_2-5. Accordingly, 95% of the inserted larvae maturated to adults in the control, fulfilling the guideline requirements, and for all test concentrations (nominal) from 1.0 to 3.2 mg a.s./L high emergence rates of between 87 to 92% were recorded. At test concentrations (nominal) of 5.6 and 10 mg a.s./L no emergence could be observed. The χ^2-test (p = 0.05) established no statistical significant difference in numbers of emerged midges per sex at all test concentration. Since no statistical significant difference was found for all vessels of test concentrations with successful emergence a dose-related effect can be excluded. Because it is not possible to introduce the same number of female and male organisms as larvae into each beaker, the emergence rates of male and female numbers are pooled for the statistical analysis. The start of emergence was on day 14 to 15 for test concentrations (nominal) from 1.0 and 3.2 mg a.s./L and for the control. The mean development time and rate were calculated for each beaker. At test concentration of 3.2 mg a.s./L the mean development time was 14% higher compared to the control. Increasing test substance concentrations inhibit the mature of the larvae.
5.3	**Conclusion**	The EC_{15} (28 d) for *Chironomus riparius* was determined to be 2.51 mg a.s./L.
5.3.2	Reliability	[redacted]
5.3.2	Deficiencies	[redacted]

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA, XIII.3.4 *Chironomus riparius*

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	June 2004
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA, XIII.3.4 *Chironomus riparius*

[redacted]

Reliability [redacted]

Acceptability [redacted]

Remarks [redacted]

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA, XIII.3.4 *Chironomus riparius*

	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*

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA, XIII.3.4 *Chironomus riparius*

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_2-1: Dilution water

Criteria	Details
Source	Test and breeding water were prepared as "M7-medium". The medium is prepared using deionised water and adding mineral salts and vitamins. The concentrations of them in the water are given in table 1 of the report.
Alkalinity	53.4 mg/L $CaCO_3$
Hardness	213.6 mg/L $CaCO_3$
pH	8.1
Ca / Mg ratio	-
Na / K ratio	-
Oxygen content	8.9 mg/L
Conductance	594 µS/cm
Holding water different from dilution water	No

Table A7_4_1_2-1a: Test sediment

Sediment characterisation	Details
Particle size distribution (USDA-Norm)	Sand: 77.1% Silt: 12.2% Clay: 10.7%
Organic carbon (%)	2.0
Water content (%)	29.9
pH	6.9
Cation exchange capacity (meq/100 g sediment)	10.5

Table A7_4_1_2-2: Test organisms

Criteria	Details
Strain	*Chironomus riparius*, first larval stage (L1)
Source	Test specimen of *Chironomus riparius* were obtained from a culture maintained at the University of Sheffield (UK) in autumn 1991 and kept in an in-house laboratory since then.
Age (at start of the study)	1st instars < 2-3 days old
Breeding method	2-4 egg masses are placed into a prepared basin. The hatched larvae are fed with green algae and an aqueous suspension of a vegetable fish food (TetraPhyll®). After 2-3 weeks the adults emerge. After mating, female adults will lay egg masses on the water surface where these can be taken to start a

Criteria	Details
	new culture or to perform toxicity tests. The culture conditions are 20 ± 2 °C and 16:8 hours light-dark-cycle. The L1 larvae used in the study were obtained by introducing some fresh egg masses in small dishes with culture medium. Two to three days after hatching, the L1 larvae were transferred with a blunt pipette to the test vessel.
Kind of food	During the study the test organisms were fed with a commercial ornamental fish food extract (TetraPhyll®) (aqueous suspension, 1 g TetraPhyll®/20 ml water) as used for the breeding.
Amount of food	About 1 mg TetraPhyll®/larvae/day
Feeding frequency	During the study the larvae were fed at least about three times per week.
Pretreatment	One day prior to treatment (day –1) the test organisms were transferred in a randomised procedure into the test containers.
Feeding of animals during test	Yes, The amount of the suspension was added to each of the test container on days: -1, 0, 1, 2, 3, 6, 7, 8, 9, 10, 13, 14, 15, 16, 17, 20, 21, 22, 23, 24 and 25

Table A7_4_1_2-3: Test system

Criteria	Details
Static test	The bottom of the test containers were covered with a 1.5 cm high layer of sediment. 0.38 L water were slowly poured into the beakers. The height of the water was 6.0 cm.
Volume of test vessels	0.6 L glass beakers with an average diameter of 9.5 cm.
Volume water/animal	19 ml
Number of animals/vessel	20
Number of vessels/ concentration	3 beakers per test concentration and control
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_2-4: Test conditions

Criteria	Details
Test temperature	20 ± 2 °C
Dissolved oxygen	Control: 6.6 (minimum at the end) – 9.0 (maximum at day –1); Test concentrations: 6.8 (minimum at the end) - 9.1 (maximum at day –1)
pH	Control: 8.1 (minimum at the end) – 8.7 (maximum at day –1); Test concentrations: 7.8 (minimum at the end) – 8.8 (maximum at day –1)
Adjustment of pH	No
Aeration of dilution water	Yes, the water was aerated and temperated to 20 °C in an in-house preparation tank. (The aeration was stopped for 24-hours after insertion of the test organism). Gentle aeration in the test containers was provided through a glass Pasteur pipette situated about 2.5 cm above the sediment layer.
Quality/Intensity of irradiation	Light intensity was on average about 1021 lux
Photoperiod	16:8 light-dark-cycle

Table A7_4_1_2-5: Numbers of emerged midges over 28 days

Summary of numbers of emerged midges over 28 days					
Initial nominal conc. (mg a.s./L)	No. of inserted larvae	No. of emerged midges	Emergence (%) of inserted larvae	% male of emergence	% female of emergence
Control	60	[redacted]	[redacted]	[redacted]	[redacted]
1.0	60	[redacted]	[redacted]	[redacted]	[redacted]
1.8	60	[redacted]	[redacted]	[redacted]	[redacted]
3.2	60	[redacted]	[redacted]	[redacted]	[redacted]
5.6	60	[redacted]	[redacted]	[redacted]	[redacted]
10	60	[redacted]	[redacted]	[redacted]	[redacted]

Table A7_4_1_2-6: Influence of tebuconazole on the emergence and development of *Chironomus riparius* after 28 days (based on initial measured concentrations)

[mg a.s./L]	**EC_{15}**	95 % confidence limits	EC_5	EC_{10}	EC_{50}
Emergence ratio (pooled by sex)	**2.51**	2.24 – 2.82	2.37	2.45	2.78

Table A7_4_1_2-7 a: **Emergence data (sum of three replicates)**

Test-Substance Concentration [mg a.s./l]		Emerged midges (total number of inserted larvae: 60)											
		Number						Percentage					
		21 days			28 days			21 days			28 days		
nominal	measured	M	F	Total	M	F	Total	M	F	Total	M	F	Total
Control	<0.052												
1.0	0.729												
1.8	1.31												
3.2	2.33												
5.6	4.08												
10	7.29												

M: Male. F: Female. Total: Sum of male and female midges

Table A7_4_1_2-7b: **Mean development time and rate (mean of three replicates)**

Test-Substance Concentration [mg a.s./l]		Mean development time and rate of fully emerged midges (male and female midges pooled)			
		Development time (d)		Development rate (d^{-1})	
nominal	measured	Average	±SD	Average	±SD
Control	<0.052				
1.0	0.729				
1.8	1.31				
3.2	2.33				
5.6	4.08				
10	7.29				

-: No emergence observed at nominal concentrations of 5.6 and 10 mg a.s./l

Table A7_4_1_2-7c: **Effect data,** 28 days (confidence limits) in initial measured concentrations [mg/l]

	EC_5	EC_{10}	EC_{15}	EC_{50}
Emerged midges				
Development rate (F)				
Development rate (M)				
Development rate (M+F)				

[1] indicate if effect data are based on nominal (n) or measured (m) concentrations

Section A7.4.3.5.1 Effects on any other specific, non-target organisms believed to be at risk

Annex Point IIIA XIII 3.4

Chironomus riparius

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		1 REFERENCE
1.1	**Reference**	Heimbach F. (1996), Influence of Tebuconazole (tech.) on Development and Emergence of Larvae of *Chironomus riparius* in a Water-Sediment System, BAYER AG, Leverkusen, Germany. Report No. HBF/Ch 10.
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	"Yes" No guidelines available, however this study was don according to a Proposal for a BBA-Guideline: "Effects of plant protection products on the development of sediment-dwelling larvae of *Chironomus riparius* in a water-sediment system"
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]
		3 MATERIALS AND METHODS
3.1	**Test material**	Tebuconazole (tech.) As given in section 2
3.1.1	Lot/Batch number	batch number [redacted]
3.1.2	Specification	As given in section 2
3.1.3	Purity	[redacted] active substance
3.1.4	Composition of Product	
3.1.5	Further relevant properties	
3.1.6	Method of analysis	Not described
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	See table A7_4_3_5_1-1

Section A7.4.3.5.1 Effects on any other specific, non-target organisms believed to be at risk

Annex Point IIIA XIII 3.4

Chironomus riparius

3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	
3.4	**Testing procedure**	
3.4.1	Dilution water Test Sediment	details on dilution water see table A7_4_3_5_1-2
3.4.2	Test organisms	details on tested organisms see table A7_4_3_5_1-3
3.4.3	Test system	Further details on test type, renewal of TS solution, laboratory equipment, loading, replicates etc. see table A7_4_3_5_1-4
3.4.4	Test conditions	This study was conducted as limit test with only one test concentration, 0.1 mg AI/l. Further relevant test conditions in tabular form see table A7_4_3_5_1-5
3.4.5	Duration of the test	28 days
3.4.6	Test parameter	The sex, time and number of emerged adults
3.4.7	Sampling	The test vessels were observed at least three times per week to make a visual assessment of any behavioural differences compared to the control. The sex, time and number of emerged adults were recorded daily during the period of emergence.
3.4.8	Monitoring of TS concentration	Yes Chemical analysis of overlying water and filtrate from sediment (= pore water) 1 hour, 7 and 28 days after application
3.4.9	Statistics	Statistical analysis was obtained by employing a computerized program χ^2-test was performed to establish different sensitivities of sexes, and ANOVA for emergence Rate.
	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	This study was conducted as limit test with only one test concentration, 0.1 mg AI/l.

Section A7.4.3.5.1 Effects on any other specific, non-target organisms believed to be at risk

Annex Point IIIA XIII 3.4

Chironomus riparius

4.2.2 Actual concentrations of test substance

Nominal	Analytical results of tebuconazole					
	1 hour/day 0		Day 7		Day 28	
	Conc mg AI/l	% of initial nominal	Conc. mg AI/l	% of initial conc	Conc mg AG/l	% of initial conc
	Overlying water					
0.1mg/l	0.099	99	0.065	65	0.011	11
	Pore water					
0.1 mg/l	<0.01	---	0.012	---	0.01	---

4.2.3 Effect data

Numbers of emerged midges				
Initial conc. mg AI/l	No. of emerged midges	Emergence (%) of inserted larvae	% male of emergence	% female of emergence
Control	140	93.3	46.4	53.6
Solvent conc	142	94.7	47.9	52.1
0.1	131	87.3	45.8	54.2

Average mean development time (d) was 16.5 ± 0.26 for control, 15.9 ± 0.48 for solvent control, and 16.2 ± 0.52 for midges exposed for 0.1 mg AI/l.

4.2.4 Concentration / response curve4.

4.2.5 Other effects — No

4.3 Results of controls — See above

4.4 Test with reference substance — Not performed

4.4.1 Concentrations -

4.4.2 Results -

Section A7.4.3.5.1
Annex Point IIIA XIII 3.4

Effects on any other specific, non-target organisms believed to be at risk
Chironomus riparius

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The test is performed as limit test with only one test concentration, 0.1 mg AI/l, according to the proposed BBA-method "Effects of plant protection products on the development of sediment-dwelling larvae of *Chironomus riparius* in a water-sediment system", January 1996.

5.2 Results and discussion

During the study, the concentration of Tebuconazole (tech.) in the test water and pore water was analysed day 0 (one h after the start), day 7, and day 28. The analytical results of day 0 indicated that 99% of the initial nominal concentration. The concentration of active ingredient in the overlying water declined continuously during the study. On day 7, 65% of nominal initial were found and 11% on day 28. Only a small amount of the active ingredient was detected in the pore water (less than 1%). These results indicate that some part of the test substance adsorbed to the sediment and/or was degraded during the study.

The %-emergence of midges in the control in relation to the number of inserted larvae was high and fulfilled the guideline requirements: 93.3% of the inserted larvae maturated to adults. The mean development time of the test organisms was less than 20 days (16.5 days in the control).

The number of emerged midges and the time of emergence were not influenced at the test concentration of 0.1 mg AI/l (U-test, p 0 0.05). Also the day of first emergence was not postponed.

5.3 Conclusion

The test is valid and performed according to the proposed BBA-method "Effects of plant protection products on the development of sediment-dwelling larvae of *Chironomus riparius* in a water-sediment system", January 1996.

5.3.2 Reliability [redacted]

5.3.2 Deficiencies [redacted]

Section A7.4.3.5.1 Effects on any other specific, non-target organisms believed to be at risk

Annex Point IIIA XIII 3.4

Chironomus riparius

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*02 04 04*
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	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*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_4_3_5_1-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	Yes Acetone
Concentration of vehicle	54.7 mg test substance was given ad 50 ml acetone. Of this acetone solution, 25 ml was made up to 2000-ml test water (= stock solution). To reach the initial nominal test concentration of 0.1 mg/AI/l, 20 ml of the stock solution were applied to the water in the test chambers
Vehicle control performed	Yes For the solvent control, 6.25-ml acetone was made up to 500 ml test water and 20 ml of this solution were applied to each test vessel.
Other procedures	

Table A7_4_3_5_1-2: Dilution water

Criteria	Details
Source	M7-medium (is similar to the M4-medium for daphnia
Alkalinity ($CaCO_3$)	54 mg/l
Hardness ($CaCO_3$)	196 mg/l
pH	7.8
Ca / Mg ratio	
Na / K ratio	
Oxygen content	10 ppm
Conductance	560 µS/cm
Holding water different from dilution water	No

Table A7_4_3_5_1-2a: Sediment

Sediment characterisation	Details
Particle size distribution (USDA-Norm)	Sand: 78.1% Silt: 9.3% Clay: 12.6%
Organic carbon (%)	5.0
Water holding capacity (%)	100.9 (= 502 g/kg d.w. sediment)
PH	5.8
Cation exchange capacity	10.0 (meq/100 g sediment)

Table A7_4_3_5_1-3: Test organisms

Criteria	Details
Strain	*Chironomus riparius*
Source	In-house culture.
Age (at start of the study)	Two to 3 days after hatching the L1-larvae were transferred to the test vessels.
Breeding method	2-4 egg masses are placed into the prepared basin. The hatched larvae are fed with green algae and an aqueous suspension of a vegetable fish food (Tetraphyll). After 2 –3 weeks the adults emerge. After mating, female adults will lay egg masses on the water surface where these can be taken to start a new culture or to perform toxicity tests.
Kind of food	Tetraphyll
Amount of food	About 1 mg Tetraphyll/larvae/day
Feeding frequency	Tetraphyll was added to each test container on days: -1, 0, 1, 3, 6, 8, 10, 13, 15, 17, 21, and 23.
Pretreatment	Non
Feeding of animals during test	Yes, see above

Table A7_4_3_5_1-4: Test system

Criteria	Details
Static test	The test sediment used was artificial sediment which was prepared 11 days before the start of the test. The bottoms of the test container were covered with a 2-cm high layer of sediment. The beakers were filled with 2.65 l water. The height of the water was 20 cm.
Volume of test vessels	3 l glass beakers with a diameter of 13.5 cm
Volume water/animal	106 ml
Number of animals/vessel	25
Number of vessels/ concentration	For biological evaluations 6 replicates were prepared of each treatment
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_3_5_1-5: Test conditions

Criteria	Details
Test temperature	20 °C +/- 2 °C
Dissolved oxygen	9 mg/l(start) – 5.8 mg/l (minimum at the end)
PH	6.6 (min at the end) – 7.7 (start)
Adjustment of pH	No
Aeration of dilution water	Yes Gentle aeration was provided through a glass Pasteur pipette situated about 2.5 cm above the sediment layer.
Quality/Intensity of irradiation	Light intensity was about 1200 lux
Photoperiod	16 h daylight – 8 h dark

Section A7.4.3.5.2 Aquatic plant toxicity

Annex Point IIIA, XIII.3.4 *Lemna gibba*

			Official use only
		1 REFERENCE	
1.1	**Reference**	Bowers, L.M. (1997): Toxicity of Folicur Technical to *Lemna gibba* G3. Bayer Corporation, Agricultural Division, Environmental Research Section, Stilwell, Kansas, USA, Report No. 107681, unpublished. Date: 1997-03-13.	
1.2	**Data protection**	[redacted]	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes; US-EPA FIFRA Guideline 123-2 (Growth and Reproduction of Aquatic Plants, Tier 2)	
2.2	**GLP**	[redacted]	
2.3	**Deviations**	[redacted]	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Tebuconazole (Folicur technical)	
3.1.1	Lot/Batch number	Batch number [redacted]	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	Not applicable	
3.1.5	Further relevant properties	Water solubility = 32 mg/l at 20 °C	
3.1.6	Method of analysis	The analytical report with detailed description of the analytical method for determination of Tebuconazole in water is attached to the test report (pp. 29 ff.)	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	See table A7_4_3_5_2-1	
3.3	**Reference substance**	No reference substance investigated.	
3.3.1	Method of analysis for reference	Not applicable	