

Section A7.5.1.2 Earthworm, acute toxicity test**Annex Point IIIA XIII 3.2***Eisenia fetida andrei*

			Official use only
1 REFERENCE			
1.1 Reference		Heimbach, F., 1989, Toxicity of Euparen® (WG) to Earthworms, Bayer AG, Institute for Environmental Biology, Leverkusen, Germany, 1987-09-18.	
1.2 Data protection		Yes	
1.2.1 Data owner		Bayer Crop Science AG	
1.2.2 Companies with letter of access		Bayer Chemicals AG	
1.2.3 Criteria for data protection		Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
2 GUIDELINES AND QUALITY ASSURANCE			
2.1 Guideline study		Yes	
		OECD Guideline No. 207 (April 1984)	
2.2 GLP		Yes	
2.3 Deviations		No	
3 METHOD			
3.1 Test material		Euparen 50 WG	
3.1.1 Lot/Batch number		Batch Number ██████████	
3.1.2 Specification		██████ dichlofluanid, water dispersible granule	
3.1.3 Purity		██████ of active substance	X
3.1.4 Composition of Product		Euparen WG 50 is a granule formulation and applied in agriculture as fungicide. It contains ████████ dichlofluanid	X
3.1.5 Further relevant properties		-	
3.1.6 Method of analysis		No data	
3.2 Reference substance		Yes; chloroacetamide	
3.2.1 Method of analysis for reference substance		No data	
3.3 Testing procedure			
3.3.1 Preparation of the test substance		I. Pre-Test: 1.25 g of Euparen WG 50 were weighed, 250 ml of deionised water was added and stirred for two hours. II. For Main Study 8.9 g of Euparen WG 50 were weighed ad 1000 ml of deionised water and stirred for one hour.	X
3.3.2 Application of the test substance		The test substance was added into 5 g quartz sand and pounded well. From these mixtures, the concentrations were produced for the study by mixing into the test substrate thoroughly with a domestic mixer. At the same time, 100 ml deionised water were mixed into the test substrate in	

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		each test container. 500 g dry weight test substrate (equivalent to 775 g wet weight) was prepared for each test container.
3.3.3	Test organisms	See table A7_5_1_2-2
3.3.4	Test system	See table A7_5_1_2-3
3.3.5	Test conditions	See table A7_5_1_2-4
3.3.6	Test duration	14 days
3.3.7	Test parameter	Mortality and weight alteration of the survivors
3.3.8	Examination	Seven days after the start of the study, the number of surviving earthworms was counted by emptying the substrate out onto an inert surface and removing the earthworms by hand. The animals were then returned to the test container with the substrate. After 14 days the weight of surviving earthworms was determined as well as their number. Earthworms which show no reaction upon being prodded with a blunt probe are considered dead.
3.3.9	Monitoring of test substance concentration	No
3.3.10	Statistics	The weight alterations of the test organisms were statistically evaluated by the U-Test of Wilcoxon, Mann & Whitney (Sachs, L. (1978): <i>Angeordnete Statistik</i> , Springer Verlag, Heidelberg, New York), Probability level $P = 0.05$, two sided). As in the pre-test the number of replicates was too low ($n = 1$) the U-test was only performed in the main test.

4 RESULTS

4.1	Filter paper 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	Soil test		
4.2.1	Initial concentrations of test substance	See table A7_5_1_2-3	
4.2.2	Effect data (Mortality)	For mortalities and weight alterations see table A7_5_1_2-5; the ecotoxicological endpoints are reported in table A7_5_1_2-6.	X
4.2.3	Concentration / effect curve	Regression curve (after Litchfield & Wilcoxon) for dichlofluanid was not calculated. For the reference substance the line of regression (after Litchfield & Wilcoxon) had a gradient of $s = 1.32$	
4.2.4	Other effects	The weight alterations of the surviving animals are given in table A7_5_1_2-5	

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- 4.3.1 Mortality See Table A7 5 1 2-5
- 4.3.2 Number/percentage of earthworms showing adverse effects No adverse effects observed
- 4.3.3 Nature of adverse effects No adverse effects observed

4.4 Test with reference substance

Yes;
chloroacetamide

- 4.4.1 Concentrations 10, 18, 24, 32 and 56 mg/kg
- 4.4.2 Results LC₅₀ (14 days) = 22.8 mg/kg dry weight substrate (95% confidence limits 21.3-24.4 mg/kg). This value is within the concentration range normally determined in international ring studies

5 APPLICANT'S SUMMARY AND CONCLUSION**5.1 Materials and methods**

Acute earthworm toxicity of Euparen WG 50 (a.i. dichlofluanid) was investigated according to OECD Guideline 207. No significant deviations from the guideline. The test animals were exposed to following concentrations of Euparen WG 50 (in mg formulation/kg dry weight substrate): 0.1, 1, 10, 100 and 1000 (pre-test) and 100, 562, 1000 and 1780 (main test), respectively. After 14 days, the number of surviving animals and their weight alteration was determined.

5.2 Results and discussion

- 5.2.1 LC₀ 288 mg a.i. / kg dry weight substrate
- 5.2.2 LC₅₀ > 913 mg a.i./ kg dry weight substrate

5.3 Conclusion

The mortality rate in the control was below 10% which is regarded as the limit for natural mortality. The properties of the substrate are in agreement with the nominal values. The LC₅₀ of the reference substance is within the usual range. The test conditions are therefore equivalent to the standard.

X

5.3.1 Other Conclusions -

5.3.2 Reliability 1

5.3.3 Deficiencies

Yes;
Information incomplete about the composition of Euparen WG 50;
physical-chemical properties of the dilution water not given

X

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Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPporteur MEMBER STATE
Date	03/08/06
Materials and Methods	Accept applicant's version, the UK CA notes that: 3.1.3 Purity is only [REDACTED] dichlofluanid 3.1.4 The information given about the composition of Euparen WG 50 is incomplete, this is identified as a deficiency by the applicant in 5.3.3. Some further information related to Euparen WG 50 has been provided subsequently, and is located in the Confidential Annex of the dossier. 3.3.1 There is no reference to the table A7_5_1_2-1 with information about the test substance preparation. There are no details about the dilution water, as identified by the applicant in 5.3.3.
Results and discussion	Accept applicant's version, the UK CA notes that: 4.2.2 Body weight data available in study and used in Document IIA by applicant would have been useful if presented. 5.2 Weight alterations were observed in the earthworms from the test concentration below the concentrations with mortalities. Therefore, the NOEC value calculated on the basis of weight alterations was 51.3 and not 288 mg a.s./kg dry weight substrate as stated in the report.
Conclusion	Accept applicant's version
Reliability	Reliability = 2
Acceptability	Acceptable The use of a test substance with only [REDACTED] dichlofluanid is considered a minor deficiency for this study.
Remarks	The concentrations used in the study were nominal and as the applicant identifies this is in line with the guideline. All endpoints and data presented in the summary and tables have been checked against the original summary and are correct.
	COMMENTS FROM ... (specify)
Date	<i>Give date of comments submitted</i>
Materials and Methods	<i>Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state</i>
Results and discussion	<i>Discuss if deviating from view of rapporteur member state</i>
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>
Reliability	<i>Discuss if deviating from view of rapporteur member state</i>
Acceptability	<i>Discuss if deviating from view of rapporteur member state</i>

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Remarks

Table A7_5_1_2-1: Preparation of TS solution

Criteria	Details
Type and source of dilution water	deionised water
Alkalinity / Salinity	-
Hardness	-
PH	-
Oxygen content	-
Conductance	-
Holding water different from dilution water	No

Table A7_5_1_2-2: Test organisms

Criteria	Details
Species/strain	<i>Eisenia fetida andrei</i>
Source of the initial stock	Strain of Prof. Graff, Federal German Biological Agency for Agriculture and Forestry (BBA), Brunswick, Germany
Culturing techniques	The animals are kept at 22 ± 2 °C, 70-90% relative humidity, 12:12 hour light-dark cycle. The substrate consists of ca. 70% by weight of natural soil, 25% peat and 5% straw (dry weight in each case). The animals are fed on ground, dried cattle manure at 14 day intervals. At the same time, the substrate is also replenished with water. The animals are transferred into fresh substrate at half-yearly intervals.
Age/weight	The adult worms used in the test were more than two months old. Average weight at the start of the study was 317 mg in the pre-test and 340 mg in the main study.
Pre-treatment	On the day prior to the start of the study, the earth-worms were removed from the breeding substrate for acclimatisation and kept in the test substrate (without test substance) under the test conditions until the start of the study.

Table A7_5_1_2-3: Test system

Criteria	Details
Artificial soil test substrate	The test substrate consists of 69% fine quartz sand (84% of the sand has a particle size of 0.06-0.2 mm), 10% dried, finely ground peat (sphagnum peat; pH 2-4), 20% kaolin (kaolinite content of around 36%, pH value ca. 7) and around 1% calcium carbonate (pure) to adjust the pH value to 6 +/- 0.5. The substrate was first of all mixed dry from these components in a mixer, and moistened with water. When adding the test substance, 100 ml deionised water was also added to each test container so that the water content was around 35% when the worms were introduced.
Test mixture	Not applicable
Size, volume and material of test container	1.5 litre preserving jars, covered with glass lids
Amount of artificial soil (kg)/ container	500 g dry weight (equivalent to 775 g wet weight)
Nominal levels of test concentrations	I. Pre-Test: Control, 0.1, 1, 10, 100 and 1000 mg Euparen WG 50/kg dry weight substrate II. Main Test: Control, 100, 562, 1000 and 1780 mg Euparen WG 50/kg dry weight substrate
Number of replicates/concentration	4
Number of earthworms/test concentration	40
Number of earthworms/container	10
Light source	Constant light 400-800 lux
Test performed in closed vessels due to significant volatility of test substrate	No

Table A7_5_1_2-4: Test conditions

Criteria	Details
Test temperature	20 ±1 °C
Moisture content	Moisture content in substrate [%] / [% of max. water capacity]: Pre-test: Start of study: 25.8 / 52.5; End of study: 35.1 / ---; Main test: Start of study: 26.2 / 57.8; End of study: 33.4 / ---
pH	Pre-test: Start of study: 6.27; End of study: 6.47; Main test: Start of study: 5.80; End of study: 5.90
Adjustment of pH	Yes; Around 1% pure calcium carbonate was added to the test substrate to adjust the pH value to 6.0 ± 0.5
Light intensity / photoperiod	Constant light (400 – 800 lux)
Relevant degradation products	Degradation products were not investigated in this study.

Table A7_5_1_2-5: Mortality data and weight alteration of the test animals

Nominal Test Substance Concentration [mg Euparen WG 50/kg dry weight substrate]	Mortality				Weight alteration of the survivors	
	Number		%		%	U-test (P = 0.05)
	after 7 d	after 14 d	after 7 d	after 14 d		
I. PRE-TEST						
Control		0		0	-4	
0.1		0		0	+1	
1		0		0	+3	
10		0		0	+3	
100		0		0	±0	
1000		28		70	-12	
II: MAIN TEST						
Control	0	1	0	3 ±5	+3 ±2	-
100	0	0	0	0	+1 ±1	-
562	0	0	0	0	-14 ±5	-
1000	3	3	8 ±5	8 ±5	-18 ±5	+
1780	8	8	20 ±12	20 ±12	-14 ±6	0

*: Results of the U-test: + = weights of control and the test concentration do differ significantly; - = weights of control and the test concentration do differ significantly

Table A7_5_1_2-6: Effect data after 14 days (nominal concentrations)

		[mg Euparen WG 50/kg dry weight substrate]	[mg a.i./kg dry weight substrate]
LC50		> 1780	> 913
LLC	lowest lethal conc.	1780	913
LOEC	lowest observed effect conc.	1000	513
NOEC (LC0)	no-observed-effect-conc.	562	288

Table A7_5_1_2-7: Validity criteria for acute earthworm test according to OECD Guideline 207

	fulfilled	Not fulfilled
Mortality of control animals < 10%	X	