

**Section 7.5.1.3 Terrestrial plant toxicity**  
**Annex Point IIIA XIII 3.4**

		Official use only
		X
<b>1 REFERENCE</b>		
<b>1.1 Reference</b>	Pallett, K., Gosch, H. 2004, Effects of Dichlofluanid on the phytotoxicity of non-target plants: seedling, emergence and seedling growth test. Bayer CropScience GmbH, Ecotoxicology, Industriepark Hoechst, Frankfurt/M., study identification SE04/004, 2004-09-21.	
<b>1.2 Data protection</b>	Yes	
1.2.1 Data owner	Bayer Chemicals AG	
1.2.2 Companies with letter of access	-	
1.2.3 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
<b>2 GUIDELINES AND QUALITY ASSURANCE</b>		
<b>2.1 Guideline study</b>	Yes OECD 208 A (July 2000, draft)	
<b>2.2 GLP</b>	Yes	
<b>2.3 Deviations</b>	Test starts when 65% of the plants were emerged instead of 50% (regarded as minor deviation). Tier I test with 100 mg/kg soil.	X
<b>3 METHOD</b>		
<b>3.1 Test material</b>	Dichlofluanid, colourless white crystals	
3.1.1 Lot/Batch number	██████████	
3.1.2 Specification	The test substance was identified by an Certificate of Analysis from July 11, 2002 (expiry date: July 2006). Project Number of the Standard Certificate of Analysis: ██████████	
3.1.3 Purity	Purity ██████	
3.1.4 Composition of Product	Not applicable	
3.1.5 Further relevant properties		
3.1.6 Method of analysis		
<b>3.2 Preparation of TS solution for poorly soluble or volatile test substances</b>	1) Mechanical mixing of 1500 mg solid test material (a.i.) in 1 kg soil (dry weight) to a pre-mixture (stock mixture 1) 2) Mixing 1 kg of the stock mixture into 15 kg soil to a concentration of 100 mg a.i. per kg soil (dry weight)	
<b>3.3 Reference substance</b>	No reference substance was used	
3.3.1 Method of analysis for reference substance	-	
<b>3.4 Testing procedure</b>		

### Section 7.5.1.3 Terrestrial plant toxicity

#### Annex Point IIIA XIII 3.4

3.4.1	Dilution water	Not used due to the instability of dichlofluanid in water.
3.4.2	Test plants	see A7_5_1_3-1
3.4.3	Test system	see table A7_5_1_3-2
3.4.4	Test conditions	see table A7_5_1_3-2
3.4.5	Test duration	The tests were started when at least 65% of the seedlings had emerged (= Day 0) and were finished 14 days after this date
3.4.6	Test parameter	Effects on seedling emergence, survival (mortality), phytotoxicity, growth stages at the final assessment and biomass (shoot dry weight) determined 14 days after emergence of 65% of seeds in the controls
3.4.7	Sampling	-
3.4.8	Method of analysis of the plant material	Visual
3.4.9	Quality control	OK
3.4.10	Statistics	For data evaluation, the mean values per plant at the different concentrations were calculated as percentage of untreated plants and the related standard deviation were assessed. Significant differences to the control value were identified by a Williams-test.

## 4 RESULTS

### 4.1 Results test substance

4.1.1	Applied initial concentration	100 mg/kg soil dry weight (nominal)
4.1.2	Phytotoxicity rating	<b>Oilseed rape:</b> Only slight visual phytotoxic symptoms were observed in oilseed rapes (10 % deviation from the control, marginal necrosis at the edges of some leaves). <b>Soybean and Oats:</b> No significant effects were seen.
4.1.3	Plant height	-
4.1.4	Plant dry weights	<b>Oilseed rape:</b> A significant reduction of 29 % in comparison to the control was seen in biomass (dry weight) <b>Soybean:</b> A 5 % reduction in comparison to the control was seen in biomass (dry weight). This is not statistically significant according to the Williams t-test. <b>Oats:</b> An 8 % reduction in comparison to the control was seen in biomass (dry weight). This is not statistically significant according to the Williams t-test.
4.1.5	Root dry weights	-
4.1.6	Root length	-
4.1.7	Number of dead plants	related to emerged plants: <b>Oilseed rape:</b> none (0/39) <b>Oats:</b> none (0/40) <b>Soybean:</b> two (2/35)

4.1.8	Effect data on emergence 14 days after emergence of 65 % of the seeds in the controls	<p><b>Oilseed rape:</b> 92.3 % of the control (36/39)</p> <p><b>Oats:</b> 100 % of the control (40/40)</p> <p><b>Soybean:</b> 102.9 % of the control (36/35)</p>	
4.1.9	Concentration / response curve	not applicable, single dose test.	
4.1.10	Other effects	None	
<b>4.2 Results of controls</b>			
4.2.1	Number/ percentage of plants showing adverse effects	No adverse effects were reported from the controls	
4.2.2	Nature of adverse effects	-	
<b>4.3 Test with reference substance</b>			
4.3.1	Concentrations	-	
4.3.2	Results	-	
<b>5 APPLICANT'S SUMMARY AND CONCLUSION</b>			
5.1	<b>Materials and methods</b>	OECD 208A (draft July 2000). No deviations from this guideline	X
5.2	<b>Results and discussion</b>	<p>Oats (<i>Avena sativa</i>)</p> <p>The application of 100 mg/kg dichlofluanid incorporated into the soil had no impact on <b>emergence</b> or <b>survival</b> of oats. Biomass, in terms of <b>dry weight</b>, was reduced by 8% by the test item however this was not significant at the 95% confidence limits with the Williams t-test. There were no phytotoxic symptoms with resulting from the test item and there was no adverse impact on plant growth.</p> <p>Oilseed rape (<i>Brassica napus</i>)</p> <p>The application of 100 mg/kg dichlofluanid incorporated into the soil resulted in a 7.7% inhibition of <b>emergence</b> of rape. There was no adverse effect on <b>survival</b>. Biomass, in terms of <b>dry weight</b>, was reduced by 29% that was significant at the 95% confidence limits with the Williams t-test. There was some minor phytotoxicity visible as a necrosis at the leaf edges however, this was recorded as less than 10% and had no adverse impact on plant growth.</p> <p>Soybean (<i>Glycine max</i>)</p> <p>The application of 100 mg/kg dichlofluanid incorporated into the soil had no impact on <b>emergence</b> of soybean. Two plants that emerged did not survive within the assessment period leading to 5.6% <b>mortality</b>. Biomass, in terms of <b>dry weight</b>, was reduced by 7% by the test item however this was not significant at the 95% confidence limits with the Williams t-test. There were no phytotoxic symptoms with resulting from the test item and there was no adverse impact on plant growth.</p>	

5.2.1	EC <sub>20</sub>	Cannot be determined from a single dose test. The highest effect during the test was 29 % related to biomass reduction of oilseed rape.
5.2.2	EC <sub>50</sub>	An EC <sub>50</sub> was not reached during the test. Nevertheless for risk assessment purposes the application rate of 100 mg /kg can be regarded as a (worst case) EC <sub>50</sub>
5.2.3	EC <sub>80</sub>	Not applicable
<b>5.3</b>	<b>Conclusion</b>	Based on the results of this study in which Dichlofluanid was tested under glasshouse conditions adverse effects were observed however none exceed the 50% adverse effect trigger to merit the next tier for Non-target terrestrial plant studies.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

X

### Evaluation by Competent Authorities

Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

#### EVALUATION BY RAPporteur MEMBER STATE

<b>Date</b>	28/01/05
<b>Materials and Methods</b>	Accept applicant's version noting the following:  As identified by the applicant in 2.3 the test starts when 65% of the plants were emerged instead of 50% as in the guideline. This is a minor deficiency, so there is a deficiency, which is not indicated in 5.1 and 5.3.2.
<b>Results and discussion</b>	Accept applicant's version
<b>Conclusion</b>	Accept applicant's version
<b>Reliability</b>	Reliability = 1
<b>Acceptability</b>	Acceptable
<b>Remarks</b>	The only deficiency is considered to be minor and not effect the validity of the result. The guideline is adhered to in all other respects.  The UK CA had to request a full revision of this summary by the Applicant during the evaluation stage due to many drafting errors. Therefore, the lack of comments by the UK CA is due to earlier concerns being addressed. All endpoints and data presented in the summary and tables have been checked against the original summary and are correct.

#### COMMENTS FROM ... (specify)

<b>Date</b>	<i>Give date of comments submitted</i>
<b>Materials and Methods</b>	<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>
<b>Results and discussion</b>	<i>Discuss if deviating from view of rapporteur member state</i>
<b>Conclusion</b>	<i>Discuss if deviating from view of rapporteur member state</i>
<b>Reliability</b>	<i>Discuss if deviating from view of rapporteur member state</i>
<b>Acceptability</b>	<i>Discuss if deviating from view of rapporteur member state</i>
<b>Remarks</b>	

Table A7\_5\_1\_3-1: Test plants

	Family	Species	Common name	Source (seed/plant)
<b>Dicotyledonae</b>	Brassicaceae	<i>Brassica napus</i>	Oilseed rape	Commercial sources
	Fabaceae	<i>Glycine max</i>	Soybean	Commercial sources
<b>Monocotyledonae</b>	Gramineae	<i>Avena sativa</i>	Oat	Commercial sources

Table A7\_5\_1\_3-2: Test system

Criteria	Details
Test type	Glasshouse conditions
Container type	Plastic pots (10 cm in diameter)
Seed germination potential	Rate of emergence in the controls: Oat = 100% Oilseed rape = 97.5% Soybean = 87.5%
Identification of the plant species	-
Number of replicates	8
Numbers of plants per replicate per dose	5 seeds were sowed in each replicate.
Date of planting	February 13, 2004
Plant density	Five plants per replicate (plastic pots 10 cm in diameter)
Date of test substance application	February 13, 2004
High of plants at application	The test seeds were sowed in soil incorporated with the test item
Date of phytotoxicity rating or harvest	At days 7 and 14 phytotoxic symptoms were assessed (e.g. stunted growth, discoloration, necrosis).
Dates of analysis	-
Test type	Terrestrial plants, growth test according to OECD 208A draft (pre-test)
Method of application	The active substance was mixed into the soil.
Application levels	-
Dose rates	One concentration equivalent to 100 mg test item per kg soil (dry weight) plus untreated controls.
Substrate characteristics	Soil Type: Standard soil (silty loam) from Bayer CropScience, sieved to 2 mm PH 7.4 Organic carbon (g/100 g dry soil)%: 1.19

Table Table A7\_5\_1\_3-2: Test system -continued

Criteria	Details
Watering of the plants	Initial top watering to facilitate germination was followed by bottom watering for the rest of the test.
Temperature	The test plants were grown at $25 \pm 3$ °C during daytime and $18 \pm 5$ °C at night (minor deviations up to 32 °C and down to 11.5 °C occurred for short times and do not have effects on the plant growth).
Thermoperiod	-
Light regime	16 h light : 8 h dark
Relative humidity	-
Wind volatility	-
Observation periods and duration of test	<p>The number of plants emerged per replicate was recorded on daily until 65% was reached.</p> <p>On days 7 and 14 phytotoxic symptoms were assessed.</p> <p>Determination of plant dry weight was carried out at the end of the test (Day 14) for all plants of one pot as one replicate.</p>
Pest control	Sterilisation of the soil
Any other treatments and procedures	Fertilisation of the soil