**Table A7_4_1_2-2: Test organisms**

| Criteria | Details |
|---|---|
| Strain | *Chironomus riparius*, animals of the first larval stage (L1) were used |
| Source | [redacted] |
| Age (at start of the study) | 1st instars < 2-3 days old |
| 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.<br>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 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 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,<br>The amount of the suspension was added to each of the test container on days: -1, 0, 3, 4, 5, 6, 7, 10, 11, 12, 13, 14, 17, 18, 19, 20, 21, 24, 25, 26 and 27 |

**Table A7_4_1_2-3: Test system**

| Criteria | Details |
|---|---|
| Static test | The bottom of the test containers were covered with a 2 cm high layer of sediment. 2.65 l water were slowly poured into the beakers. The height of the water was 20 cm. |
| Volume of test vessels | 3 l glass beakers with an average diameter of about 13.5 cm |
| Volume water/animal | 106 ml |
| Number of animals/vessel | 25 |
| Number of vessels/ concentration | 1-2 replicates for each test substance concentration, 3 replicates were prepared for the 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:<br>9.1 (minimum at the end) – 9.8 (maximum at day –1);<br>Test concentrations:<br>9.1 (minimum at the end) - 9.9 (maximum at day –1) |
| pH | Control:<br>5.1 (minimum at the end) – 7.8 (day –1);<br>Test concentrations:<br>5.0 (minimum at the end) – 7.9 (maximum at day –1);<br>The pH-values decreased during the study in all test beakers of the control and the treatments except of the highest test concentration. |
| Adjustment of pH | No |
| Aeration of dilution water | Yes,<br>the water was aerated and temperated to 20 °C in an in-house preparation tank.<br>(The aeration was stopped for 24-hours after insertion of the test organism).<br>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 2000 lux |
| Photoperiod | 16:8 light-dark-cycle with a 30 minutes dusk and dawn period. |

**Table A7_4_1_2-5: Effect data**

| Summary of numbers of emerged midges over 28 days | | | | | |
|---|---|---|---|---|---|
| Nominal conc. (mg pure metabolite/l | No. of inserted larvae | No. of emerged midges | Emergence (%) of inserted larvae | % male of emergence | % female of emergence |
| Control | 75 | 61 | 81 | 41 | 59 |
| 0.010 | 25 | 20 | 80 | 40 | 60 |
| 0.032 | 25 | 19 | 76 | 37 | 63 |
| 0.10 | 50 | 38 | 76 | 37 | 63 |
| 0.32 | 50 | 39 | 78 | 46 | 54 |
| 1.0 | 50 | 39 | 78 | 38 | 62 |
| 3.2 | 50 | 43 | 86 | 44 | 56 |
| 10 | 50 | 37 | 74 | 41 | 59 |
| 32 | 25 | 18 | 72 | 56 | 44 |
| 100 | 25 | 0 | - | - | - |

**Table A7_4_1_2-6: Influence on the development after 28 days (based on nominal concentrations)**

| | $EC_{15}$ (mg pure metabolite/l) | 95 % c.l. (mg pure metabolite/l) | $EC_5$ (mg pure metabolite/l) | $EC_{10}$ (mg pure metabolite/l) | $EC_{50}$ (mg pure metabolite/l) |
|---|---|---|---|---|---|
| Emergence rate (pooled sex) | 31.1 | 23.5 - 41.1 | 24.4 | 28.2 | 46.9 |
| Development rate (pooled sex) | 16.7 | not calculable | 9.7 | 13.4 | 42.3 |
| Development rate (male) | 13.8 | not calculable | 7.4 | 10.7 | 40.6 |
| Development rate (female) | 17.2 | not calculable | 10.1 | 13.9 | 43.1 |

The day of first emergence was postponed for about one day at the test concentration 10 mg pure metabolite/l and for two days at 32 mg pure metabolite/l.

**Table A7_4_1_2-7: Analytical results of dimethylaminosulfanilid**

| Nominal conc. (mg pure metabolite/l) | Analytical results of dimethylaminosulfanilid, means of two analyses each (mg pure metabolite/l) | | | | | |
|---|---|---|---|---|---|---|
| | 1 hour/day 0 | | day 7 | | day 28 | |
| | Conc. | % of nominal conc. | Conc. | % of nominal conc. | Conc. | % of nominal conc. |
| | Overlying water | | | | | |
| Control | < 0.01 | - | n.a. | - | n.a. | - |
| 0.10 | 0.09 | 90 | 0.09 | 90 | 0.08 | 80 |
| 1.0 | 0.94 | 94 | 0.92 | 92 | 0.79 | 79 |
| 10 | 9.7 | 97 | 9.1 | 91 | 8.4 | 84 |
| | Pore water | | | | | |
| Control | < 0.01 | - | n.a. | - | n.a. | - |
| 0.10 | 0.01 | 0.58 | 0.06 | 3.31 | 0.08 | 4.74 |
| 1.0 | 0.11 | 0.57 | 0.55 | 3.26 | 0.68 | 4.05 |
| 10 | 1.1 | 0.68 | 5.8 | 3.33 | 7.4 | 4.36 |

n.a.: not analysed

## Section A7.5.1.1 Inhibition to microbial activity (terrestrial)

**Annex Point IIA7.4**

| | | | Official use only |
|---|---|---|---|
| | | **1 REFERENCE** | |
| **1.1** | **Reference** | a) [redacted] 1991, Influence of the Commercial Product ®Euparen WG 50 on the Soil Respiration after Amendment with Glucose [redacted]<br>b) [redacted] 1991, Influence of the Commercial Product ®Euparen WG 50 on the Microbial Mineralization of Carbon in Soils [redacted]<br>[redacted] 1991, Influence of the Commercial Product ®Euparen WG 50 on Nitrogen Mineralization in Soil [redacted] | |
| **1.2** | **Data protection** | Yes | |
| 1.2.1 | Data owner | [redacted] | |
| 1.2.2 | Companies with letter of access | [redacted] | |
| 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,<br>all studies were carried out according to the Guidelines for the Official Testing of Plant Protectants, Part VI, Influence on the activity of the Soil Microflora, BBA Braunschweig, Germany, March 1990. | |
| **2.2** | **GLP** | Yes | |
| **2.3** | **Deviations** | No | |
| | | **3 MATERIALS AND METHODS** | |
| **3.1** | **Test material** | dichlofluanid formulation: Euparen WG 50 | |
| 3.1.1 | Lot/Batch number | 233715493 | |
| 3.1.2 | Specification | [redacted]ichlofluanid, water dispersible granule | X |
| 3.1.3 | Purity | [redacted] dichlofluanid | |
| 3.1.4 | Composition of Product | [redacted]dichlofluanid | X |
| 3.1.5 | Further relevant properties | - | |
| 3.1.6 | Method of analysis | a) For $CO_2$ in the respiration test: The quantities of $CO_2$ were measured after absorption in NaOH and following titration (Gas analyzer: Wösthoff Co., Bochum, Germany)<br>b) The $CO_2$ released from the soil was drawn through 40 ml 0.5 M | |

## Section A7.5.1.1 Inhibition to microbial activity (terrestrial)

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| | | | |
|---|---|---|---|
| | | NaOH by means of $CO_2$-free air at a rate of 60 ml/min. The lye was replaced weekly and the amount of bound $CO_2$ was determined by titration with 0.1 M HCl from pH 8.3 to pH 3.8. An automatic titrator with sample changer was used for these analyses.<br>c) For the determination of the nitrification: Three photometric methods were used to measure ammonium (colour complex at 660 nm), nitrate plus nitrite (after nitrate reduction and formation of an azo dyestuff at 540 nm) and nitrite (also at 540 nm). Determination with Technicon Autoanalyzer II. | |
| **3.2** | **Reference substance** | No | X |
| 3.2.1 | Method of analysis for reference substance | - | |
| **3.3** | **Testing procedure** | | |
| 3.3.1 | Soil sample / inoculum / test organism | See table A7_5_1_1-1 | X |
| 3.3.2 | Test system | See table A7_5_1_1-2 | |
| 3.3.3 | Application of TS | See table A7_5_1_1-3 | |
| 3.3.4 | Test conditions | See table A7_5_1_1-4 | |
| 3.3.5 | Test parameter | a) Respiration Test: Inhibition of microbial carbon transformation<br>b) Mineralisation Test: Inhibition of microbial mineralisation of lucerne-grass-green-meal<br>c) Nitrification Test: Inhibition of nitrification of ammonia | |
| 3.3.6 | Analytical parameter | a) Respiration Test: $CO_2$ measurement<br>b) Mineralisation Test: $CO_2$ measurement<br>c) Nitrification Test: Ammonia and nitrate (including nitrite) measurement | |
| 3.3.7 | Duration of the test | a) Respiration Test: 91 days<br>b) Mineralisation Test: 91 days<br>c) Nitrification Test: 91 days | |
| 3.3.8 | Sampling | See table A7_5_1_1-3 | |
| 3.3.9 | Monitoring of TS concentration | No | |
| 3.3.10 | Controls | Carricr (quartz sand) control | |
| 3.3.11 | Statistics | a) Averages and standard deviations of the 3 soil samples per evaluation day were calculated;<br>b) Averages and standard deviations of the 3 soil samples per evaluation day were calculated;<br>c) Averages and standard deviations of the 3 soil samples per evaluation day were calculated; t-Test with 5% probability level | |

## Section A7.5.1.1 Inhibition to microbial activity (terrestrial)

**Annex Point IIA7.4**

| | | | |
|---|---|---|---|
| | | was used to evaluate significant differences between treated and untreated soil samples in nitrogen mineralization. | |
| | | **4 RESULTS** | |
| **4.1** | **Range finding test** | Not performed | |
| 4.1.1 | Concentration | n.a. | |
| 4.1.2 | Effect data | n.a. | |
| **4.2** | **Results test substance** | | |
| 4.2.1 | Initial concentrations of test substance | In all studies: 0, 6.7 and 67 mg Euparen 50 WG/kg dry weight soil. These application rates were equivalent to 5 and 50 kg Euparen WG 50/ha which is equivalent to the recommended agricultural field dose and a 10-fold overdose (calculated according a method given by German BBA) | X |
| 4.2.2 | Actual concentrations of test substance | Since the soil was not extracted and analyzed, values given for the active ingredients are nominal | X |
| 4.2.3 | Growth curves | n.a. | |
| 4.2.4 | Cell concentration data | n.a. | |
| 4.2.5 | Concentration/ response curve | n.a. | |
| 4.2.6 | Effect data | The data show, that the product did not cause a change in soil pH.<br>a) Respiration Test: See table A7_5_1_1-5. During the 91-day experiments, 6.7 mg Euparen WG 50/kg dry wt soil had no meaningful influence on respiration after adding glucose (3000 mg/kg dry wt soil) to loamy sand and loamy silt. In contrast to this, a 10-fold overdose (67 mg Euparen WG 50/kg dry wt soil) caused a reduction in the amount of glucose degraded. When applied as recommended under practical conditions, Euparen WG 50 will not influence carbon turnover in soil.<br>b) Mineralisation Test: See table A7_5_1_1-6. During the 91-day experiments, 6.7 mg and also 67 mg Euparen WG 50/kg dry wt soil had no influence on the mineralisation of lucerne-grass-green-meal in a loamy sand and loamy silt. When applied as recommended under practical conditions, Euparen WG 50 will not affect carbon transformations in soil.<br>c) Nitrification Test: See table A7_5_1_1-7. During the 91-day experiments, 6.7 mg Euparen WG 50/kg dry wt soil had no influence on nitrogen mineralisation in loamy sand and loamy silt. In contrast to this, a 10-fold overdose (67 mg Euparen WG 50/kg dry wt soil) induced a temporary inhibition and, subsequently, a temporary stimulation of nitrogen mineralisation in both soils. After 91 days, there were no differences between treated and untreated soils. When applied as recommended under practical conditions, Euparen WG 50 will not affect nitrogen mineralisation in soil. | |
| 4.2.7 | Other observed effects | - | |

## Section A7.5.1.1 Inhibition to microbial activity (terrestrial)

**Annex Point IIA7.4**

| | | | |
|---|---|---|---|
| **4.3** | **Results of controls** | See table A7_5_1_1-5 (Respiration Test), table A7_5_1_1-6 (Mineralisation Test) and table A7_5_1_1-7 (Nitrification Test). | |
| **4.4** | **Test with reference substance** | No reference substance investigated. | |
| 4.4.1 | Concentrations | - | |
| 4.4.2 | Results | - | |
| | | **5 APPLICANT'S SUMMARY AND CONCLUSION** | |
| **5.1** | **Materials and methods** | The influence of Euparen WG 50 (a.i. [redacted]% dichlofluanid) on the soil carbon turnover and transformation on and soil nitrification was investigated. Used concentrations: 0, 6.7 and 67 mg Euparen WG 50/kg dry weight soil; these application rates were equivalent to 5 and 50 kg Euparen WG 50/ha which is equivalent to the recommended agricultural field dose and a 10-fold overdose (calculated according a method given by German BBA).<br><br>All studies were carried out according to the Guidelines for the Official Testing of Plant Protectants, Part VI, Influence on the activity of the Soil Microflora, BBA Braunschweig, Germany, March 1990. | |
| **5.2** | **Results and discussion** | The data show, that the product did not cause a change in soil pH.<br>a) **Respiration Test:** See table A7_5_1_1-5. During the 91-day experiments, 6.7 mg Euparen WG 50/kg dry wt soil had no meaningful influence on respiration after adding glucose (3000 mg/kg dry wt soil) to loamy sand and loamy silt. In contrast to this, a 10-fold overdose (67 mg Euparen WG 50/kg dry wt soil) caused a reduction in the amount of glucose degraded. When applied as recommended under practical conditions, Euparen WG 50 will not influence carbon turnover in soil.<br>b) **Mineralisation Test:** See table A7_5_1_1-6. During the 91-day experiments, 6.7 mg and also 67 mg Euparen WG 50/kg dry wt soil had no influence on the mineralisation of lucerne-grass-green-meal in a loamy sand and loamy silt. When applied as recommended under practical conditions, Euparen WG 50 will not affect carbon transformations in soil.<br>c) **Nitrification Test:** See table A7_5_1_1-7. During the 91-day experiments, 6.7 mg Euparen WG 50/kg dry wt soil had no influence on nitrogen mineralisation in loamy sand and loamy silt. In contrast to this, a 10-fold overdose (67 mg Euparen WG 50/kg dry wt soil) induced a temporary inhibition and, subsequently, a temporary stimulation of nitrogen mineralisation in both soils. After 91 days, there were no differences between treated and untreated soils. When applied as recommended under practical conditions, Euparen WG 50 will not affect nitrogen mineralisation in soil. | X |
| 5.2.1 | NOEC | n.a. | |
| 5.2.2 | $EC_{10}$ | n.a. | |
| 5.2.3 | $EC_{50}$ | n.a. | |
| **5.3** | **Conclusion** | When applied as recommended under practical conditions, Euparen WG | X |

## Section A7.5.1.1 Inhibition to microbial activity (terrestrial)

**Annex Point IIA7.4**

| | | |
|---|---|---|
| | | 50 will not influence carbon turnover, carbon transformations and nitrogen mineralisation in soil. |
| 5.3.1 | Reliability | Reliability indicator: 2 |
| 5.3.2 | Deficiencies | Yes;<br>Information incomplete about the composition of Euparen WG 50 |

| Evaluation by Competent Authorities | |
|---|---|
| | Use separate "evaluation boxes" to provide transparency as to the comments and views submitted |

**EVALUATION BY RAPPORTEUR MEMBER STATE**

**Date** 28/01/05

**Materials and Methods** [redacted]

**Results and discussion** [redacted]

**Conclusion** [redacted]

**Reliability**

**Acceptability**

## Section A7.5.1.1 Inhibition to microbial activity (terrestrial)

**Annex Point IIA7.4**

| | |
|---|---|
| **Remarks** | All endpoints and data presented in the summary and tables have been checked against the original summary and are correct. |
| | **COMMENTS FROM ...** |
| **Date** | *Give date of comments submitted* |
| **Materials and Methods** | *Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.*<br>*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_5_1_1-1: Properties of soil samples**

| Criteria | Details | |
|---|---|---|
| Nature | Loamy sand | Loamy silt |
| Sampling site: | Soil sample from Germany | Soil sample from Germany |
| Geographical reference on the sampling site | [redacted] | [redacted] |
| Data on the history of the site | Plant protection chemicals have not been used on the field since 1981; grass was planted in 1987, summer wheat in 1988, winter rye in 1988/89 and winter barley in 1989/90. | Plant protection chemicals have not been used on the field since 1981; 19851986/87 winter barley, 1987/88 oat, 1988 winter barley, spring 1989 perennial ryegrass. |
| Use pattern | Agricultural soil | Agricultural soil |
| Depth of sampling [cm] | Not reported | Not reported |
| Sand / Silt / Clay content [% dry wt] | 69.1 / 22.4 / 8.5 (loamy sand) | 5.4 / 82.5 / 12.1 (loamy silt) |
| pH (1 M KCl) | 4.42-4.60 | 6.25-6.29 |
| Organic carbon content [% dry wt] | 0.84 | 1.75 |
| Nitrogen content [% dry wt] | 0.08 | 0.18 |
| Cation exchange capacity [meq/100 g dry wt soil] | Not reported | Not reported |
| Initial microbial biomass [mg microbial C/kg dry wt soil] | 155 | 597 |
| Reference of methods | J.P.E. Anderson, 1982, Soil Respiration, in: Page, A.L. et al. (eds.): Methods of Soil Analysis, Part 2 (Chemical and Microbiological Methods), Agronomy Monograph 9, 2nd ed., Madison, USA, pp. 831-871 | |
| Collection / storage of samples | The soils were sampled from the field, passed through a sieve (2 mm) and stored until used, as described in ISO/DIS 1036-6 (1992) | |
| Preparation of inoculum for exposure | n.a. | |
| Pretreatment | n.a. | |

**Table A7_5_1_1-2: Test system for soil respiration / nitrification tests**

| Criteria | Details |
|---|---|
| Culturing apparatus | a) Respiration Test: after mixing soil samples equivalent to 750 g dry wt soil were poured into 1000 ml preserving jars with glass lids (without rubber rings).<br>b) Mineralisation Test: after mixing soil samples equivalent to 100 g dry wt soil were poured into 500 ml brown glass bottles with screw cup aeration system.<br>c) Nitrification Test: treated soil samples (weight not given) were poured into 500 ml brown glass bottles with screw cup aeration system. |
| Number of vessels / concentration | 3 |
| Aeration device | No |
| Measuring equipment | No |
| Test performed in closed vessels | No |

**Table A7_5_1_1-3: Application of test substance and sampling**

| Criteria | Details |
|---|---|
| Application procedure | Addition of pre-mixtures in a carrier and mixing the carrier with native soil |
| Carrier | Quartz sand |
| Concentration of liquid carrier [% v/v] | No liquid carrier |
| Liquid carrier control | n.a. |
| Sampling procedure | a) Respiration Test: Moist samples (equivalent to 25 g dry wt. soil) were taken from each treatment on day 0 (within 3 hours after treatment), and after 14, 28, 41 or 42, 56, 70 and 91 days of incubation. The samples were mixed with glucose, poured into plastic cylinders and connected to the gas analyzer.<br>b) Mineralisation Test: The lye (1 M NaOH) containers in the incubation bottles were sampled and replaced weekly (day 0, 7, 14, 21, 28, 42, 56, 70 and 91).<br>c) Nitrification Test: Immediately after treatment and after 7, 13 or 14, 21, 28, 42, 56 or 57, 70 and 91 days, the soil in each jar was mixed with a spatula and a moist sample (equivalent to 10 g dry wt. soil) was extracted with 50 ml 1 M KCl; after filtration the extracts were analyzed on an autoanalyser. |

**Table A7_5_1_1-4: Test conditions**

| Criteria | Details |
|---|---|
| Organic (inorganic) substrate | a) Respiration Test: addition of 3000 mg glucose/kg dry wt. soil, to induce a maximum respiration rate in soil<br>b) Mineralisation Test: Addition of 5000 mg lucerne grass-green meal/kg dry wt. soil, to induce a maximum respiration rate in soil<br>c) Nitrification Test: addition of 5000 mg lucerne grass-green meal/kg dry wt. soil, to stimulate nitrogen stimulation in soil |
| Incubation temperature | 20 ± 2 °C |
| Soil moisture | 45-48% of the water holding capacity |
| Method of soil incubation | Bulk |
| Aeration | No |

**Table A7_5_1_1-5A: Respiration in a loamy sand soil 14 after treatment with Euparen WG 50 and addition of glucose (3000 mg/kg dry wt soil)**

| Hours after addition of Glucose | 0 mg Euparen WG 50/kg[1] (quartz sand only) | 6.7 mg Euparen WG 50/kg | 67 mg Euparen WG 50/kg[1] |
|---|---|---|---|
| | mg carbon dioxide/hour/kg dry wt soil (average ± standard deviation) | | |
| **2** | 5.66 ± 1.17 | 5.35 ± 0.58 | 2.48 ± 0.25 |
| **3** | 5.56 ± 1.06 | 5.35 ± 0.57 | 2.54 ± 0.42 |
| **4** | 5.66 ± 1.06 | 5.49 ± 0.41 | 2.50 ± 0.30 |
| **5** | 5.70 ± 1.09 | 5.66 ± 0.51 | 2.56 ± 0.18 |
| **6** | 6.00 ± 1.13 | 5.58 ± 0.56 | 2.54 ± 0.23 |
| **7** | 6.06 ± 0.93 | 6.04 ± 0.39 | 2.63 ± 0.17 |
| **8** | 6.57 ± 0.91 | 6.08 ± 0.53 | 2.58 ± 0.31 |
| **9** | 6.64 ± 1.03 | 6.38 ± 0.41 | 2.78 ± 0.10 |
| **10** | 7.24 ± 0.85 | 7.02 ± 0.28 | 2.84 ± 0.07 |
| **11** | 7.80 ± 0.80 | 7.19 ± 0.47 | 2.79 ± 0.19 |
| **12** | 8.15 ± 1.14 | 7.93 ± 0.30 | 3.06 ± 0.12 |
| **13** | 8.97 ± 0.72 | 8.93 ± 0.16 | 3.09 ± 0.11 |
| **Sum** | 80.00 | 76.99 | 32.38 |
| **% of Control** | 100.0 | 96.2 | 40.5 |

1 = dry wt soil / average from three samples

**Table A7_5_1_1-5B: Respiration in a loamy silt soil 14 after treatment with Euparen WG 50 and addition of glucose (3000 mg/kg dry wt soil)**

| Hours after addition of Glucose | 0 mg Euparen WG 50/kg[1] (quartz sand only) | 6.7 mg Euparen WG 50/kg | 67 mg Euparen WG 50/kg[1] |
|---|---|---|---|
| | mg carbon dioxide/hour/kg dry wt soil (average ± standard deviation) | | |
| 2 | 28.47 ± 2.45 | 27.93 ± 1.58 | 23.78 ± 0.54 |
| 3 | 28.50 ± 0.48 | 28.13 ± 0.47 | 22.60 ± 0.25 |
| 4 | 29.85 ± 0.55 | 26.93 ± 1.84 | 23.52 ± 1.41 |
| 5 | 29.05 ± 0.53 | 28.73 ± 0.33 | 23.62 ± 0.40 |
| 6 | 30.53 ± 0.30 | 30.84 ± 0.47 | 24.43 ± 0.31 |
| 7 | 33.03 ± 0.89 | 33.01 ± 0.73 | 25.70 ± 0.50 |
| 8 | 36.87 ± 0.29 | 36.67 ± 0.41 | 27.46 ± 1.12 |
| 9 | 41.42 ± 0.18 | 40.10 ± 0.34 | 30.94 ± 1.95 |
| 10 | 46.10 ± 0.61 | 46.48 ± 0.95 | 35.75 ± 1.25 |
| 11 | 54.12 ± 0.47 | 55.79 ± 0.70 | 41.64 ± 1.53 |
| 12 | 66.37 ± 0.95 | 65.17 ± 0.34 | 49.93 ± 3.25 |
| 13 | 77.44 ± 1.97 | 75.81 ± 1.62 | 62.56 ± 2.67 |
| Sum | 501.75 | 495.60 | 391.93 |
| % of Control | 100.0 | 98.8 | 78.1 |

1 = dry wt soil / average from three samples

**Table A7_5_1_1-6: Influence of Euparen WG 50 on the microbial mineralisation of lucerne-grass-green-meal in a loamy sand and a loamy silt soil**

| Days after Treatment | 0 mg Euparen WG 50/kg[1] (quartz sand only) | 6.7 mg Euparen WG 50/kg | 67 mg Euparen WG 50/kg[1] |
|---|---|---|---|
| | mg carbon dioxide/100 g dry wt soil (average ± standard deviation) | | |
| | LOAMY SAND SOIL | | |
| 7 | 227.8 ± 3.9 | 219.6 ± 15.5 | 208.3 ± 11.3 |
| 14 | 55.7 ± 3.8 | 67.1 ± 6.6 | 74.0 ± 3.4 |
| 21 | 25.3 ± 1.3 | 28.9 ± 1.2 | 36.4 ± 1.4 |
| 28 | 18.2 ± 1.6 | 19.7 ± 2.2 | 21.8 ± 1.2 |
| 42 | 22.9 ± 3.4 | 23.0 ± 2.8 | 26.7 ± 1.7 |
| 56 | 16.7 ± 4.8 | 14.5 ± 3.6 | 16.7 ± 2.7 |
| 71 | 11.6 ± 2.5 | 11.4 ± 1.8 | 14.5 ± 1.1 |
| 91 | 14.4 ± 0.9 | 14.2 ± 3.4 | 13.9 ± 1.2 |
| | LOAMY SILT SOIL | | |
| 7 | 231.3 ± 4.8 | 230.9 ± 4.8 | 218.0 ± 6.5 |
| 14 | 73.7 ± 3.8 | 75.4 ± 1.1 | 85.5 ± 3.3 |
| 21 | 43.9 ± 1.3 | 44.0 ± 0.0 | 44.2 ± 0.6 |
| 28 | 37.0 ± 1.6 | 34.6 ± 2.6 | 34.6 ± 1.9 |
| 42 | 52.8 ± 3.4 | 55.8 ± 5.7 | 48.2 ± 0.6 |
| 56 | 39.5 ± 6.3 | 43.0 ± 6.3 | 40.0 ± 2.6 |
| 70 | 31.7 ± 3.7 | 31.5 ± 3.0 | 29.4 ± 2.3 |
| 91 | 37.4 ± 3.3 | 41.2 ± 5.1 | 35.0 ± 0.9 |

1 = dry wt soil / average from three samples

**Table A7_5_1_1-7A: Nitrogen mineralization in a loamy sand soil after treatment with Euparen WG 50 and addition of lucerne-grass-green meal (5000 mg/kg dry wt soil)**

| Days after Treatment | 0 mg Euparen WG 50/kg[1] (quartz sand only) | | 6.7 mg Euparen WG 50/kg | | 67 mg Euparen WG 50/kg[1] | |
|---|---|---|---|---|---|---|
| | mg nitrogen/kg dry wt soil (average ± standard deviation) | | | | | |
| | ammonium | nitrate | ammonium | nitrate | ammonium | nitrate |
| 0 | 3.07 ± 0.35 | 11.66 ± 0.67 | 2.86 ± 0.04 | 10.91 ± 0.03 | 2.93 ± 0.18 | 11.06 ± 0.34 |
| 7 | 2.06 ± 0.38 | 2.36 ± 0.16 | 3.89 ± 0.73 (t) | 1.51 ± 0.15 (t) | 10.25 ± 0.38 (t) | 1.07 ± 0.07 (t) |
| 14 | 1.11 ± 0.04 | 11.72 ± 0.37 | 2.02 ± 0.38 | 13.30 ± 0.30 (t) | 19.55 ± 1.13 (t) | 3.07 ± 0.21 (t) |
| 21 | 1.70 ± 0.51 | 19.09 ± 1.01 | 1.56 ± 0.49 | 22.77 ± 0.44 (t) | 16.58 ± 0.86 (t) | 12.37 ± 0.43 (t) |
| 28 | 1.56 ± 0.27 | 23.66 ± 0.80 | 1.50 ± 0.28 | 29.47 ± 0.09 (t) | 8.53 ± 1.92 (t) | 28.22 ± 1.06 (t) |
| 42 | 1.17 ± 0.07 | 32.09 ± 0.33 | 1.24 ± 0.10 | 37.72 ± 0.62 (t) | 2.27 ± 0.44 | 40.10 ± 0.98 (t) |
| 57 | 0.60 ± 0.02 | 39.98 ± 2.59 | 0.52 ± 0.06 | 49.23 ± 2.50 (t) | 1.35 ± 0.25 (t) | 53.71 ± 3.83 (t) |
| 70 | 0.99 ± 0.08 | 47.01 ± 2.12 | 0.99 ± 0.01 | 53.69 ± 3.20 (t) | 1.75 ± 0.16 (t) | 56.97 ± 4.23 (t) |
| 91 | 1.22 ± 0.32 | 63.06 ± 6.12 | 1.21 ± 0.17 | 63.49 ± 7.93 | 1.57 ± 0.14 | 69.82 ± 5.66 (t) |

1 = dry wt soil / average from three samples
(t) = significant difference between treated and untreated soil samples (t-Test with 5 % probability level)

**Table A7_5_1_1-7B: Nitrogen mineralization in a loamy silt soil after treatment with Euparen WG 50 and addition of lucerne-grass-green meal (5000 mg/kg dry wt soil)**

| Days after Treatment | 0 mg Euparen WG 50/kg[1] (quartz sand only) | | 6.7 mg Euparen WG 50/kg | | 67 mg Euparen WG 50/kg[1] | |
|---|---|---|---|---|---|---|
| | mg nitrogen/kg dry wt soil (average ± standard deviation) | | | | | |
| | ammonium | nitrate | ammonium | nitrate | ammonium | nitrate |
| 0 | 4.16 ± 0.17 | 12.69 ± 0.12 | 4.07 ± 0.01 | 12.55 ± 0.21 | 4.20 ± 0.07 | 12.77 ± 0.01 |
| 7 | 1.78 ± 0.15 | 5.98 ± 0.40 | 1.70 ± 0.06 | 6.28 ± 0.50 | 8.93 ± 1.21 (t) | 9.13 ± 0.17 (t) |
| 13 | 1.79 ± 0.30 | 7.73 ± 0.84 | 1.50 ± 0.21 | 8.42 ± 0.96 | 8.75 ± 2.16 (t) | 14.40 ± 1.35 (t) |
| 21 | 1.56 ± 0.26 | 13.08 ± 0.86 | 1.45 ± 0.29 | 15.16 ± 0.83 (t) | 1.39 ± 0.08 | 28.29 ± 0.21 (t) |
| 28 | 1.53 ± 0.43 | 18.64 ± 0.66 | 1.75 ± 0.46 | 20.63 ± 1.01 (t) | 1.94 ± 0.63 | 35.98 ± 0.91 (t) |
| 42 | 1.84 ± 0.30 | 18.81 ± 0.95 | 2.00 ± 0.01 | 20.38 ± 0.42 | 1.61 ± 0.26 | 29.49 ± 0.27 (t) |
| 56 | 1.27 ± 0.22 | 42.65 ± 0.68 | 1.15 ± 0.02 | 43.12 ± 1.19 | 1.56 ± 0.08 | 54.36 ± 1.17 (t) |
| 70 | 1.43 ± 0.26 | 51.09 ± 1.06 | 1.32 ± 0.24 | 49.60 ± 2.90 | 1.39 ± 0.33 | 57.86 ± 1.15 (t) |
| 91 | 0.88 ± 0.42 | 62.91 ± 3.17 | 0.88 ± 0.35 | 59.70 ± 4.00 | 1.08 ± 0.39 | 66.64 ± 2.26 |

1 = dry wt soil / average from three samples
(t) = significant difference between treated and untreated soil samples (t-Test with 5% probability level)

## 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** | [redacted] 1989, Toxicity of Euparen® (WG) to Earthworms [redacted] | |
| **1.2** | **Data protection** | Yes | |
| 1.2.1 | Data owner | [redacted] | |
| 1.2.2 | Companies with letter of access | [redacted] | |
| 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<br>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 233 715 493 | |
| 3.1.2 | Specification | [redacted]ichlofluanid, water dispersible granule | |
| 3.1.3 | Purity | [redacted]f active substance | X |
| 3.1.4 | Composition of Product | Euparen WG 50 is a granule formulation and applied in agriculture as fungicide. It contains [redacted]ichlofluanid | X |
| 3.1.5 | Further relevant properties | - | |
| 3.1.6 | Method of analysis | No data | |
| **3.2** | **Reference substance** | Yes;<br>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.<br>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 | |

| | | | |
|---|---|---|---|
| | | 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): Angewandte Statistik, 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.<br>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 | |

## Section A7.5.1.2 Earthworm, acute toxicity test
**Annex Point IIIA XIII 3.2**

*Eisenia fetida andrei*

| | | | |
|---|---|---|---|
| **4.3** | **Results of controls** | | |
| 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;<br>chloroacetamide | |
| 4.4.1 | Concentrations | 10, 18, 24, 32 and 56 mg/kg | |
| 4.4.2 | Results | $LC_{50}$ (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 | $LC_0$ | 288 mg a.i. / kg dry weight substrate | |
| 5.2.2 | $LC_{50}$ | > 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 $LC_{50}$ of the reference substance is within the usual range. The test conditions are therefore equivalent to the standard. | |
| 5.3.1 | Other Conclusions | - | |
| 5.3.2 | Reliability | 1 | |
| 5.3.3 | Deficiencies | Yes;<br>Information incomplete about the composition of Euparen WG 50; physical-chemical properties of the dilution water not given | X |

## Section A7.5.1.2 Earthworm, acute toxicity test

**Annex Point IIIA XIII 3.2**

*Eisenia fetida andrei*

| Evaluation by Competent Authorities | |
|---|---|
| | Use separate "evaluation boxes" to provide transparency as to the comments and views submitted |
| | **EVALUATION BY RAPPORTEUR MEMBER STATE** |
| **Date** | 28/01/05 |
| **Materials and Methods** | [redacted] |
| **Results and discussion** | |
| **Conclusion** | |
| **Reliability** | |
| **Acceptability** | |
| **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.*<br>*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_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 | *Eisenia fetida andrei* |
| Source of the initial stock | [redacted] |
| 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<br>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]:<br>Pre-test:<br>Start of study: 25.8 / 52.5; End of study: 35.1 / ---;<br>Main test:<br>Start of study: 26.2 / 57.8; End of study: 33.4 / --- |
| pH | Pre-test:<br>Start of study: 6.27; End of study: 6.47;<br>Main test:<br>Start of study: 5.80; End of study: 5.90 |
| Adjustment of pH | Yes;<br>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 | |

| Section A7.5.1.3<br>Annex Point IIIA7.5.1.3 | Acute toxicity to terrestrial plants | |
|---|---|---|
| | **JUSTIFICATION FOR NON-SUBMISSION OF DATA** | Official use only |
| **Other existing data [X]**<br>**Limited exposure [...]** | **Technically not feasible [ ]** **Scientifically unjustified [ ]**<br>**Other justification [...]** | |
| **Detailed justification:** | For dichlofluanid a test on terrestrial plant toxicity (Draft OECD 208A) was performed. Tier I of the test was done with 100 mg dichlofluanid/kg soil (dry weight). At this administration in one of the three plant species effects on biomass of 29 % were seen, which did not exceed the 50 % trigger to merit the next tier for non-target terrestrial plant studies.<br><br>No further testing (tier II) on toxicity to soil non-target plants is regarded to be justified because:<br><br>a) from tier I a low effect concentration or a (worst case) EC50 with respect to the plants tested of 100 mg/kg soil can be derived,<br><br>b) dichlofluanid degrades rapidly in soil,<br><br>Furthermore, the 29% reduction in biomass in one of three species does not exceed the 50 % trigger for tier II test (dose response curve). In the draft OECD guideline 208A this trigger is related to the highest test concentration of 1000 mg/kg soil. It is regarded to be reliable to apply it also to a lower concentration because the maximal exposure concentrations from the use of the active as wood preservative are far below the 100 mg/kg concentration applied in the test. | |
| **Undertaking of intended data submission [ ]** | – | |
| | **Evaluation by Competent Authorities** | |
| | *Use separate "evaluation boxes" to provide transparency as to the comments and views submitted* | |
| **Date** | **EVALUATION BY RAPPORTEUR MEMBER STATE**<br><br>13/12/04 | |

| Section A7.5.1.3 | Acute toxicity to terrestrial plants |
|---|---|
| **Annex Point IIIA7.5.1.3** | |
| **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 7.5.1.3 Terrestrial plant toxicity
**Annex Point IIIA XIII 3.4**

| | | | Official use only |
|---|---|---|---|
| | | **1 REFERENCE** | |
| **1.1** | **Reference** | [redacted] 2004, Effects of Dichlofluanid on the phytotoxicity of non-target plants: seedling, emergence and seedling growth test. [redacted] | |
| **1.2** | **Data protection** | Yes | |
| 1.2.1 | Data owner | [redacted] | |
| 1.2.2 | Companies with letter of access | [redacted] | |
| 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<br>OECD 208 A (July 2000, draft) | |
| **2.2** | **GLP** | Yes | |
| **2.3** | **Deviations** | 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 |
| | | **3 METHOD** | |
| **3.1** | **Test material** | Dichlofluanid, colourless white crystals | |
| 3.1.1 | Lot/Batch number | 890524ELB01 | |
| 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 [redacted] | |
| 3.1.3 | Purity | Purity [redacted] % | |
| 3.1.4 | Composition of Product | Not applicable | |
| 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** | 1) Mechanical mixing of 1500 mg solid test material (a.i.) in 1 kg soil (dry weight) to a pre-mixture (stock mixture 1)<br>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) | |
| **3.3** | **Reference substance** | No reference substance was used | |
| 3.3.1 | Method of analysis for reference substance | - | |
| **3.4** | **Testing procedure** | | |

## 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 | **Oilseed rape**: Only slight visual phytotoxic symptoms were observed in oilseed rapes (10 % deviation from the control, marginal necrosis at the edges of some leaves).<br>**Soybean and Oats**: No significant effects were seen. |
| 4.1.3 | Plant height | - |
| 4.1.4 | Plant dry weights | **Oilseed rape**: A significant reduction of 29 % in comparison to the control was seen in biomass (dry weight)<br>**Soybean**: 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.<br>**Oats**: 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:<br>**Oilseed rape**: none (0/39)<br>**Oats**: none (0/40)<br>**Soybean**: two (2/35) |

| | | | |
|---|---|---|---|
| 4.1.8 | Effect data on emergence 14 days after emergence of 65 % of the seeds in the controls | **Oilseed rape**: 92.3 % of the control (36/39)<br>**Oats**: 100 % of the control (40/40)<br>**Soybean**: 102.9 % of the control (36/35) | |
| 4.1.9 | Concentration / response curve | not applicable, single dose test. | |
| 4.1.10 | Other effects | None | |
| **4.2** | **Results of controls** | | |
| 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 | - | |
| **4.3** | **Test with reference substance** | Not performed | |
| 4.3.1 | Concentrations | - | |
| 4.3.2 | Results | - | |
| | | **5 APPLICANT'S SUMMARY AND CONCLUSION** | |
| **5.1** | **Materials and methods** | OECD 208A (draft July 2000). No deviations from this guideline | X |
| **5.2** | **Results and discussion** | Oats (*Avena sativa*)<br><br>The application of 100 mg/kg dichlofluanid incorporated into the soil had no impact on **emergence** or **survival** of oats. Biomass, in terms of **dry weight,** 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.<br><br>Oilseed rape (*Brassica napus*)<br><br>The application of 100 mg/kg dichlofluanid incorporated into the soil resulted in a 7.7% inhibition of **emergence** of rape. There was no adverse effect on **survival**. Biomass, in terms of **dry weight,** 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.<br><br>Soybean (*Glycine max*)<br><br>The application of 100 mg/kg dichlofluanid incorporated into the soil had no impact on **emergence** of soybean. Two plants that emerged did not survive within the assessment period leading to 5.6% **mortality**. Biomass, in terms of **dry weight,** 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. | |

| | | | |
|---|---|---|---|
| 5.2.1 | $EC_{20}$ | 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 | $EC_{50}$ | An $EC_{50}$ 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) $EC_{50}$ | |
| 5.2.3 | $EC_{80}$ | Not applicable | |
| **5.3** | **Conclusion** | 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** |
| **Date** | 28/01/05 |
| **Materials and Methods** | Accept applicant's version noting the following:<br>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. |
| **Results and discussion** | Accept applicant's version |
| **Conclusion** | Accept applicant's version |
| **Reliability** | Reliability = 1 |
| **Acceptability** | Acceptable<br>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. |
| **Remarks** | 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)* |
| **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.*<br>*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_5_1_3-1: Test plants**

| | Family | Species | Common name | Source (seed/plant) |
|---|---|---|---|---|
| **Dicotyledonae** | Brassiceae | *Brassica napus* | Oilseed rape | Commercial sources |
| | Fabaceae | *Glycine max* | Soybean | Commercial sources |
| **Monocotyledonae** | Gramineae | *Avena sativa* | 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:<br>Oat = 100%<br>Oilseed rape = 97.5%<br>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) [redacted] sieved to 2 mm<br>PH 7.4<br>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 ± 3 °C during daytime and 18 ± 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 | The number of plants emerged per replicate was recorded on daily until 65% was reached.<br>On days 7 and 14 phytotoxic symptoms were assessed.<br>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. |
| Pest control | Sterilisation of the soil |
| Any other treatments and procedures | Fertilisation of the soil |

## Section A7.5.3.1.1 Acute oral toxicity on birds

**Annex Point IIIA XIII 1.1**

| | | | Official use only |
|---|---|---|---|
| | | **1 REFERENCE** | |
| **1.1** | **Reference** | [redacted] 1986, Acute Oral LD50 of Preventol A4-S to Bobwhite Quail [redacted] | |
| **1.2** | **Data protection** | Yes | |
| 1.2.1 | Data owner | [redacted] | |
| 1.2.2 | Companies with letter of access | [redacted] | |
| 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;<br>according to US-EPA, FIFRA Guideline, Section 163, 71-1 (1984) as well as the US-EPA Toxic Substances Control Act (TSCA) and the ASTM Standard Practice (Draft 6) "Standard Practice for Conducting Acute Oral $LD_{50}$ Tests with Avian Species" | |
| **2.2** | **GLP** | Yes | |
| **2.3** | **Deviations** | Yes;<br>The mixture of the test substance with the carrier was not analyzed for concentration, homogeneity, and stability of the test substance. | X |
| | | **3 MATERIALS AND METHODS** | |
| **3.1** | **Test material** | Dichlofluanid | |
| 3.1.1 | Lot/Batch number | Batch No. N-112/1853K [redacted] | |
| 3.1.2 | Specification | As given in section 2 of dossier | |
| 3.1.3 | Purity | [redacted] | X |
| 3.1.4 | Composition of Product | - | |
| 3.1.5 | Further relevant properties | low water solubility: 1.3 mg/l | |
| 3.1.6 | Method of analysis in the diet | Methods not mentioned, results of feed analysis conducted [redacted] Analysis of protein, moisture, fat, ash, crude fiber, carbohydrates, calories, several heavy metals, several aflatoxins, several organophosphates/organochlorine insecticides and PCB. | X |
| **3.2** | **Administration of the test substance** | See table A7_5_3_1_1-1 | |
| **3.3** | **Reference substance** | No | |

## Section A7.5.3.1.1 Acute oral toxicity on birds

**Annex Point IIIA XIII 1.1**

| | | |
|---|---|---|
| 3.3.1 | Method of analysis for reference substance | - |
| **3.4** | **Testing procedure** | |
| 3.4.1 | Test organisms | See table A7_5_3_1_1-2 |
| 3.4.2 | Test system | See table A7_5_3_1_1-3 |
| 3.4.3 | Diet | See table A7_5_3_1_1-4 |
| 3.4.4 | Test conditions | See table A7_5_3_1_1-4 |
| 3.4.5 | Duration of the test | 14 days |
| 3.4.6 | Test parameter | Mortality, toxic signs, body weight changes, feed consumption, necropsy examinations |
| 3.4.7 | Examination/ Observation | See table A7_5_3_1_1-3 |
| 3.4.8 | Statistics | Body weight and feed consumption:<br>The control group mean data was compared using t-test with $P \leq 0.05$ (Sokal, R.R. & F.J. Rohlf (1969): Biometry. Freeman & Co, San Fransisco, USA) and all treatment groups data were subjected to analysis of variance (ANOVA) with $P \leq 0.05$ (Sokal, R.R. & F.J. Rohlf (1969)). If ANOVA indicated significant differences, the mean of treated group was compared to the control group using the Williams test (Williams, D.A.: A test for differences between treatment means when several dose levels are compared with a zero dose control. Biometrics, 27, 103-117. Williams, D.A.: The comparison of several dose levels with a zero dose control. Biometrics, 28, 519-531). When a parameter mean was significantly different from the controls, that treatment was considered a toxicant effect. All statistical analysis were conducted using software supplied by SAS Institute Inc., Cary, North Carolina, USA. |
| | | **4 RESULTS** |
| **4.1** | **Limit Test / Range finding test** | Limit test was performed |
| 4.1.1 | Concentration | See data given below |
| 4.1.2 | Number/ percentage of animals showing adverse effects | See data given below |
| 4.1.3 | Nature of adverse effects | See data given below |
| **4.2** | **Results test substance** | |
| 4.2.1 | Applied concentrations | 2226 mg test substance/kg bw |
| 4.2.2 | Effect data (Mortality) | No mortality was observed during 14-day observation period. |

## Section A7.5.3.1.1 Acute oral toxicity on birds

**Annex Point IIIA XIII 1.1**

| | | |
|---|---|---|
| 4.2.3 | Body weight | See table A7_5_3_1_1-5 |
| 4.2.4 | Feed consumption | See table A7_5_3_1_1-5 |
| 4.2.5 | Concentration / response curve | Not applicable |
| 4.2.6 | Other effects | No overt clinical signs of toxicity were noted in treated birds.<br>No compound-related lesions were noted in postmortem examination of birds at study termination.<br>No test substance-related effects:<br>6 males and 6 females showed gross lesions,<br>1 male showed thickened white gizzard mucosal zone,<br>1 female showed bilaterally enlarged thyroid glands. |
| **4.3** | **Results of controls** | See table A7_5_3_1_1-5 |
| 4.3.1 | Number/ percentage of animals showing adverse effects | 1 male and 2 females showed gross lesions,<br>1 female showed gizzard erosion,<br>1 female showed thickend nodular proventriculus,<br>1 male showed thickened white gizzard mucosal zone |
| 4.3.2 | Nature of adverse effects | See data given 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** | An avian single dose $LD_{50}$ limit test was conducted to estimate the toxicity of Preventol A4-S to Bobwhite quail (*Colinus virginianus*). The test complies with US-EPA FIFRA Guideline, Section 163.71-1 (1984) as well as those of the US-EPA Toxic Substances Control Act (TSCA) and the ASTM Standard Practice (Draft 6) "Standard Practice for Conducting Acute Oral $LD_{50}$ Tests with Avian Species".<br>One group of 10 birds, 5 per sex, was given a single oral dose of 2226 mg/kg bw Preventol A4-S in corn oil. Two different groups of 10 birds, 5 per sex, were similarly dosed with corn oil only and maintained as concomitant controls. Following dosing all groups were held for a 14-day observation period. |
| **5.2** | **Results and discussion** | No mortalities were observed during the course of the study. No overt clinical signs of toxicity were noted in treated birds. Statistically significant decreases in body weight were observed between the 2226 mg/kg bw group, and the control group at day 7. No such differences were noted at test termination, suggesting recovery from toxic effects. Feed consumption data support these findings. No compound-related lesions were noted in postmortem examination of birds at study termination. |
| 5.2.1 | $LD_{50}$ | > 2226 mg test substance/kg bw |

## Section A7.5.3.1.1 Acute oral toxicity on birds

**Annex Point IIIA XIII 1.1**

| | | |
|---|---|---|
| 5.2.2 | NOEC | < 2226 mg test substance/kg bw |
| **5.3** | **Conclusion** | The mortality rate in the control was below 10%. Therefore the validity criteria for avian acute oral toxicity test according to EPA OPPTS 850.2100 are fulfilled. |
| 5.3.1 | Reliability | 1 |
| 5.3.2 | Deficiencies | No |

## Section A7.5.3.1.1 Acute oral toxicity on birds

**Annex Point IIIA XIII 1.1**

| Evaluation by Competent Authorities | |
|---|---|
| | Use separate "evaluation boxes" to provide transparency as to the comments and views submitted |
| | **EVALUATION BY RAPPORTEUR MEMBER STATE** |
| **Date** | 13/12/04 |
| **Materials and Methods** | [redacted] |
| **Results and discussion** | |
| **Conclusion** | |
| **Reliability** | |
| **Acceptability** | |
| **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.*<br>*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_5_3_1_1-1: Method of administration of the test substance**

| Carrier/Vehicle | Details |
|---|---|
| Water | No |
| Organic carrier | Yes; corn oil |
| concentration of the carrier [% v/v] | Total solution volume: 30 ml |
| Other vehicle | No |
| Function of the carrier / vehicle | Solvent for test substance |

**Table A7_5_3_1_1-*2*: Test animals**

| Criteria | Details |
|---|---|
| Species/Strain | Bobwhite quail (Colinus virginianus) |
| Source | [redacted] |
| Age (in weeks), sex and initial body weight (bw) | Age: adult animals, 22-24 weeks old;<br>Sex: males and females;<br>Mean body weights:<br>211 ±13 g (control group), 214 ± 9 g (dose group) |
| Breeding population | no data |
| Amount of food | Food and water were available ad libitum, prior to and throughout the study with the exception of the 21 hours immediately prior to dosing, during which the birds were fasted. |
| Age at time of first dosing | Age: adult animals, 22-24 weeks old |
| Health condition / medication | No prophylactic medication. |

**Table A7_5_3_1_1-3: Test system**

| Criteria | Details |
|---|---|
| Test location | Indoor, in steel brooders |
| Holding pens | galvanized steel brooders (90 x 70 x 23 cm), pelletized wood was used as cage bedding und cages were not changed during the course of the study |
| Number of animals | 30 (15 males and 15 females) |
| Number of animals per pen [$cm^2$/bird] | 5 birds of a single sex (1260 $cm^2$/bird) |
| Number of animals per dose | Two control groups, each with 5 males + 5 females, One dose group with 5 males + 5 females |
| Pre-treatment / acclimatisation | Food (Agway Gamebird Ration) and water were available ad libitum, prior to and throughout the study with the exception of the 21 hours immediately prior to dosing, during which the birds were fasted. No data about length of acclimatisation period |
| Diet during test | Food (Agway Gamebird Ration) and water were available ad libitum throughout the study. |
| Dosage levels (of test substance) | One single oral dose of 2226 mg/kg bw; total volume of test solution: 30 ml |
| Replicate/dosage level | One dose group with 5 males and 5 females; gang housed in two separate breeders |
| Feed dosing method | Orally by gavage |
| Dosing volume per application | Total solution volume: 30 ml; The test solutions were administered at a rate equal to 1% of the bird body weight. |
| Frequency, duration and method of animal monitoring after dosing | Observations for mortality and toxic signs were made twice daily for 14 days post-dosing except on weekends when only one observation per day was made; feed consumption for each group was recorded daily. At the end of the study, all surviving birds were sacrificed by $CO_2$ asphyxiation. Necropsy examinations were conducted on all surviving birds. |
| Time and intervals of body weight determination | Body weights were recorded on day 0, 7 and 14 |

**Table A7_5_3_1_1-4: Test conditions (housing)**

| Criteria | Details |
|---|---|
| Test temperature | 21.1 ± 2.2 °C. |
| Shielding of the animals | No data |
| Ventilation | No data |
| Relative huminity | 40-60% |
| Photoperiod and lighting | 8/16 hour light/dark cycle |

**Table A7_5_3_1_1-5: Average body weight change and feed consumption of animals during study**

| | | CONTROL 1 | | CONTROL 2 | | DOSE GROUP | |
|---|---|---|---|---|---|---|---|
| | | males | females | males | females | males | females |
| Mean body weight [g] | Day 0 | 215 | 206 | 209 | 215 | 211 | 217 |
| | Day 7 | 229 | 213 | 217 | 220 | 198 | 194 |
| | Day 14 | 231 | 214 | 221 | 222 | 218 | 209 |
| Daily food consumption [g/bird/day] | Day 1 | 17.0 | | | | 5.4 | |
| | Day 2 | 22.8 | | | | 7.9 | |
| | Day 3 | 8.1 | | | | 1.2 | |
| | Day 4 | 17.4 | | | | 8.0 | |
| | Day 5 | 18.7 | | | | 16.4 | |
| | Day 6 | 24.6 | | | | 26.2 | |
| | Day 7 | 15.4 | | | | 16.0 | |
| | Day 8 | 13.1 | | | | 13.4 | |
| | Day 9 | 11.1 | | | | 11.5 | |
| | Day 10 | 11.5 | | | | 12.1 | |
| | Day 11 | 17.4 | | | | 19.0 | |
| | Day 12 | 19.3 | | | | 20.4 | |
| | Day 13 | 15.4 | | | | 16.4 | |
| | Day 14 | 10.4 | | | | 11.7 | |
| | Mean | 15.9 ± 4.7 | | | | 13.3 ± 6.5 | |

## Section A7.5.3.1.2 Short-term toxicity on birds (2)

**Annex Point IIIA XIII 1.2**

| | | | Official use only |
|---|---|---|---|
| | | **1 REFERENCE** | |
| **1.1** | **Reference** | [redacted] 1986, Subacute dietary LC50 of Preventol A4-S to Mallard Ducks [redacted] | |
| **1.2** | **Data protection** | Yes | |
| 1.2.1 | Data owner | [redacted] | |
| 1.2.2 | Companies with letter of access | [redacted] | |
| 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;<br>according to US-EPA, FIFRA Guideline, Section 163, 71-2 (1984) as well as the US-EPA Toxic Substances Control Act (TSCA) and the ASTM Standard Practice (E857-81) "Standard Practice for Conducting Subacute Dietary Toxicity Tests with Avian Species" | |
| **2.2** | **GLP** | Yes | |
| **2.3** | **Deviations** | No | |
| | | **3 MATERIALS AND METHODS** | |
| **3.1** | **Test material** | Dichlofluanid | |
| 3.1.1 | Lot/Batch number | Batch No. N-112/1853K [redacted] | |
| 3.1.2 | Specification | As given in section 2 of dossier | |
| 3.1.3 | Purity | [redacted] | X |
| 3.1.4 | Composition of Product | - | |
| 3.1.5 | Further relevant properties | low water solubility: 1.3 mg/l | |
| 3.1.6 | Method of analysis in the diet | Liquid Chromatography with UV-VIS Detector, Waters Z-Module, Radial Compression Column Unit (10 cm x 8 mm, Novapack ODS, 5 µm), repeatability, reliability and recovery of a.i. were confirmed; Results of feed analysis [redacted] Analysis of protein, moisture, fat, ash, crude fiber, carbohydrates, calories, several heavy metals, several aflatoxins, several organophosphates/organochlorine insecticides and PCB. | |
| **3.2** | **Administration of the test substance** | See table A7_5_3_1_2-1 | |
| **3.3** | **Reference substance** | No | |

## Section A7.5.3.1.2 Short-term toxicity on birds (2)

**Annex Point IIIA XIII 1.2**

| | | | |
|---|---|---|---|
| 3.3.1 | Method of analysis for reference substance | - | |
| **3.4** | **Testing procedure** | | |
| 3.4.1 | Test organisms | See table A7_5_3_1_2-2 | |
| 3.4.2 | Test system | See table A7_5_3_1_2-3 | |
| 3.4.3 | Diet | See table A7_5_3_1_2-4 | |
| 3.4.4 | Test conditions | See table A7_5_3_1_2-4 | |
| 3.4.5 | Duration of the test | 8 days: 5 treatment days + 3 days post-exposure observation period | |
| 3.4.6 | Test parameter | Mortality, toxic signs, body weight changes, feed consumption, necropsy examinations | |
| 3.4.7 | Examination/ Observation | See table A7_5_3_1_2-3 | |
| 3.4.8 | Statistics | Body weight and feed consumption:<br>The control group mean data was compared using t-test with $P \leq 0.05$ (Sokal, R.R. & F.J. Rohlf (1969): Biometry. Freeman & Co, San Fransisco, USA) and all body weight gain data of treatment group was subjected to analysis of variance (ANOVA) with $P \leq 0.05$ (Sokal, R.R. & F.J. Rohlf (1969)). If ANOVA indicated significant treatment effects, the means of the treatment levels were compared to that of controls using the Williams test (Williams, D.A.: A test for differences between treatment means when several dose levels are compared with a zero dose control. Biometrics, 27, 103-117. Williams, D.A.: The comparison of several dose levels with a zero dose control. Biometrics, 28, 519-531). When a treatment mean was significantly different from the control means, that treatment was considered a toxicant effect level. All statistical analysis were conducted using software supplied by SAS Institute Inc., Cary, North Carolina, USA. | |
| | | **4 RESULTS** | |
| **4.1** | **Limit Test / Range finding test** | Limit test was performed | |
| 4.1.1 | Concentration | See data given below | |
| 4.1.2 | Number/ percentage of animals showing adverse effects | See data given below | |
| 4.1.3 | Nature of adverse effects | See data given below | |
| **4.2** | **Results test substance** | | |
| 4.2.1 | Applied concentrations | Nominal concentration in diet: 5000 ppm;<br>measured concentrations: see table A7_5_3_1_2-3 | X |
| 4.2.2 | Effect data (Mortality) | No deaths were observed in ducks fed 5000 ppm Preventol A4-S for a period of five days. | |

## Section A7.5.3.1.2 Short-term toxicity on birds (2)

**Annex Point IIIA XIII 1.2**

| | | |
|---|---|---|
| 4.2.3 | Body weight | See table A7_5_3_1_2-5 |
| 4.2.4 | Feed consumption | See table A7_5_3_1_2-5 |
| 4.2.5 | Concentration / response curve | Not applicable |
| 4.2.6 | Other effects | No clinically observable signs of toxicity were noted in Preventol A4-S treated birds. At postmortem examination it was noted that 6 of 10 treated birds had multiple pinpoint tan to red raised zones in the gizzard mucosa. Microscopic evaluation indicated these consisted of multiple mild inflammatory cell foci in the superficial keratin layer of the mucosa. Since this lesion was more common in dosed birds, this was felt to reflect a mildly irritating compound effect. |
| **4.3** | **Results of controls** | See table A7_5_3_1_2-5 |
| 4.3.1 | Number/ percentage of animals showing adverse effects | Gross lesions: 7 birds (35%);<br>Fluid filled red liver zones: 6 birds (30%);<br>Gray raised gizzard mucosal zones: 1 animal (5%);<br>Meckel's diverticulum: 1 bird (5%),<br>Multiple pinpoint raised gray gizzard mucosal zones (1 bird) |
| 4.3.2 | Nature of adverse effects | Control ducks sacrificed at study termination showed occasional agonal and/or incidental lesions. One bird had multiple pinpoint raised gray gizzard mucosal zones, which were similar microscopically to those seen in the six dosed birds. |
| **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 subacute avian dietary toxicity test was conducted to estimate the toxicity of Preventol A4-S to Mallard duck (*Anas platyrhynchos*) when exposed to the diet for a period of 5 days. The test complies with US-EPA FIFRA Guideline, Section 163.71-2 (1984) as well as those of the US-EPA Toxic Substances Control Act (TSCA) and the ASTM Standard Practice (E857-81) "Standard Practice for Conducting Subacute Dietary Toxicity Tests with Avian Species".<br><br>One group of 10 birds was fed a diet containing 5000 ppm Preventol A4-S for a period of 5 days. Two additional non-treated groups of 10 birds each were maintained as concomitant controls. All groups were maintained on Preventol A4-S free feed for a three-day observation period following the five-day exposure period. |
| **5.2** | **Results and discussion** | No mortalities were noted throughout the course of this study. No grossly observable signs of toxicity were noted. Statistically significant decreases in body weight occurred in Preventol A4-S treated birds when compared with controls; however, feed consumption data suggest this may have been the result of unpalatability or gastro-intestinal tract irritation. Postmortem examinations of Preventol A4-S treated birds indicated inflammation of gizzard mucosa; suggesting the material may |

## Section A7.5.3.1.2 Short-term toxicity on birds (2)

**Annex Point IIIA XIII 1.2**

| | | |
|---|---|---|
| | | have a mild irritative effect. No other compound-related gross lesions were noted. |
| 5.2.1 | $LC_{50}$ | > 5000 ppm test substance |
| 5.2.2 | NOEC | < 5000 ppm test substance |
| **5.3** | **Conclusion** | Two of the three validity criteria for short-term avian toxicity test according to OECD Guideline 205 are fulfilled:<br>1. The mortality rate in the control was below 10%,<br>2. Test substance concentration is > 80% of nominal concentration throughout the dosing period.<br>One criterion is not fulfilled: The lowest treatment level causing no compound-related mortality or other observable toxic effects. |
| 5.3.1 | Reliability | 1 |
| 5.3.2 | Deficiencies | No |

## Section A7.5.3.1.2 Short-term toxicity on birds (2)

**Annex Point IIIA XIII 1.2**

| Evaluation by Competent Authorities | |
|---|---|
| | Use separate "evaluation boxes" to provide transparency as to the comments and views submitted |
| | **EVALUATION BY RAPPORTEUR MEMBER STATE** |
| **Date** | 28/01/05 |
| **Materials and Methods** | [redacted] |
| **Results and discussion** | |
| **Conclusion** | |
| **Reliability** | |
| **Acceptability** | |
| **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.*<br>*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_5_3_1_2-1: Method of administration of the test substance**

| Carrier/Vehicle | Details |
|---|---|
| Water | No |
| Organic carrier | Yes, corn oil and ethanol |
| Concentration of the carrier [% v/v] | Diet preparation: Appropriate amounts of Preventol A4-S, corn oil and ethanol were combined in a 250 ml Erlenmeyer flask and added to the feed while mixing in a Hobart mixer.<br>Feed for control groups: 0 ppm Preventol A4-S, 160 g corn oil, 150 ml ethanol, 15.84 kg feed.<br>Feed for treatment group: Control: 5000 ppm Preventol A4-S (= 39.548 mg), 70 g corn oil, 150 ml ethanol, 6.89 kg feed. |
| Other vehicle | Yes, feed (Purina Gamebird Startena) |
| Function of the carrier / vehicle | Facilitation of uptake |

**Table A7_5_3_1_2-2: Test animals**

| Criteria | Details |
|---|---|
| Species/Strain | Mallard duck (*Anas platyrhynchos*) |
| Source | [redacted] |
| Age (in weeks), sex and initial body weight (bw) | At an age of 1 days the quails were obtained;<br>Sex: unknown;<br>Body weights at age of 9 days: 151-171 g |
| Breeding population | No data |
| Amount of food | Food and water were available ad libitum, prior to and throughout the study. |
| Age at time of first dosing | Age: 9 days, body weights 151-171 g |
| Health condition / medication | No prophylactic medication. |

**Table A7_5_3_1_2-3: Test system**

| Criteria | Details |
|---|---|
| Test location | Indoor, in steel brooders |
| Holding pens | galvanized steel brooders (90 x 70 x 23 cm), pelletized wood was used as cage bedding und was changed once during the study. |
| Number of animals | 30 (unknown sex) |
| Number of animals per pen [cm²/bird] | 10 birds of unknown sex (630 cm²/bird) |
| Number of animals per dose | Two control groups, each 10 birds of unkown sex, One dose group with 10 birds of unkown sex |
| Pre-treatment / acclimatisation | Acclimatisation period 7 days, birds were examined upon receipt and daily throughout the acclimatisation period. Less than 5% mortality was noted prior test initiation and all unsuitable birds (injured, deformed etc.) were eliminated from inclusion in the test. Food (Purina Gamebird Startena) and water were available ad libitum, prior to and throughout the study. |
| Diet during test | Food (Purina Gamebird Startena) and water were available ad libitum throughout the study. |
| Dosage levels (of test substance) | Nominal concentration in diet: 5000 ppm; the birds were fed for 5 days;<br>measured concentrations: 4892 ± 73 ppm (day 0, three samples taken for homogeneity analysis), 5570 ppm (sample taken on day 5 from initial feed mix for stability determination). |
| Replicate/dosage level | One dose group with 10 birds; gang housed in a breeder |
| Feed dosing method | Orally by feed |
| Dosing volume per application | The group of birds was fed a diet containing 5000 ppm a.i. for a period of 5 days, ad libitum. |
| Frequency, duration and method of animal monitoring after dosing | After 5 treatment days, birds were given control feed for 3 days (post-exposure observation period). Observations for mortality and toxic signs were made twice daily except on weekends when only one observation per day was made; feed consumption for each group was recorded daily. At the end of the study, all surviving birds were sacrificed by $CO_2$ asphyxiation. Necropsy examinations were conducted on all birds at study termination, as well as on all birds that died during the in-life phase of the study. |
| Time and intervals of body weight determination | Body weights were recorded on day 0, 5 and 8 |

**Table A7_5_3_1_2-4: Test conditions (housing)**

| Criteria | Details |
|---|---|
| Test temperature | 37.8 ± 0.5 °C. |
| Shielding of the animals | No data |
| Ventilation | No data |
| Relative huminity | No data |
| Photoperiod and lighting | 8/16 hour light/dark cycle |

**Table A7_5_3_1_2-5: Average body weight change and feed consumption of animals during study**

| | | | CONTROL 1 | CONTROL 2 | DOSE GROUP |
|---|---|---|---|---|---|
| Mean body weight [g] | Treatment days | Day 0 | 164 | 158 | 160 |
| | | Day 5 | 282 | 286 | 178 |
| | Observation day | Day 8 | 345 | 358 | 264 |
| Daily food consumption [g/bird/day] | Treatment days | Day 1 | 44 | | 18 |
| | | Day 2 | 42 | | 23 |
| | | Day 3 | 46 | | 35 |
| | | Day 4 | 45 | | 30 |
| | | Day 5 | 61 | | 35 |
| | Observation days | Day 6 | 68 | | 72 |
| | | Day 7 | 59 | | 59 |
| | | Day 8 | 46 | | 47 |
| | | Mean | 51 | | 40 |

**Table A7_5_3_1_2-6: Validity criteria for short-term avian toxicity test according to OECD Guideline 205**

| | fulfilled | Not fulfilled |
|---|---|---|
| Mortality of control animals < 10% | X | |
| Test substance concentration > 80% of nominal concentration throughout the dosing period | X | |
| Lowest treatment level causing no compound-related mortality or other observable toxic effects | | X |

## Section A7.5.3.1.2 Short-term toxicity on birds (1)

**Annex Point IIIA XIII 1.2**

| | | | Official use only |
|---|---|---|---|
| | | **1 REFERENCE** | |
| **1.1** | **Reference** | [redacted] 1986, Subacute dietary LC50 of Preventol A4-S to Bobwhite Quail [redacted] | |
| **1.2** | **Data protection** | Yes | |
| 1.2.1 | Data owner | [redacted] | |
| 1.2.2 | Companies with letter of access | [redacted] | |
| 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;<br>according to US-EPA, FIFRA Guideline, Section 163, 71-2 (1984) as well as the US-EPA Toxic Substances Control Act (TSCA) and the ASTM Standard Practice (E857-81) "Standard Practice for Conducting Subacute Dietary Toxicity Tests with Avian Species" | |
| **2.2** | **GLP** | Yes | |
| **2.3** | **Deviations** | No | |
| | | **3 MATERIALS AND METHODS** | |
| **3.1** | **Test material** | Dichlofluanid | |
| 3.1.1 | Lot/Batch number | Batch No. N-112/1853K [redacted] | |
| 3.1.2 | Specification | As given in section 2 of dossier | |
| 3.1.3 | Purity | [redacted] | X |
| 3.1.4 | Composition of Product | - | |
| 3.1.5 | Further relevant properties | low water solubility: 1.3 mg/l | |
| 3.1.6 | Method of analysis in the diet | Liquid Chromatography with UV-VIS Detector, Waters Z-Module, Radial Compression Column Unit (10 cm x 8 mm, Novapack ODS, 5 µm), repeatability, reliability and recovery of a.i. were confirmed; Results of feed analysis [redacted] Analysis of protein, moisture, fat, ash, crude fiber, carbohydrates, calories, several heavy metals, several aflatoxins, several organophosphates/organochlorine insecticides and PCB. | |
| **3.2** | **Administration of the test substance** | See table A7_5_3_1_2-1 | |
| **3.3** | **Reference substance** | No | |

## Section A7.5.3.1.2 Short-term toxicity on birds (1)

**Annex Point IIIA XIII 1.2**

| | | | |
|---|---|---|---|
| 3.3.1 | Method of analysis for reference substance | - | |
| **3.4** | **Testing procedure** | | |
| 3.4.1 | Test organisms | See table A7_5_3_1_2-2 | |
| 3.4.2 | Test system | See table A7_5_3_1_2-3 | |
| 3.4.3 | Diet | See table A7_5_3_1_2-4 | |
| 3.4.4 | Test conditions | See table A7_5_3_1_2-4 | |
| 3.4.5 | Duration of the test | 8 days: 5 treatment days + 3 days post-exposure observation period | |
| 3.4.6 | Test parameter | Mortality, toxic signs, body weight changes, feed consumption, necropsy examinations | |
| 3.4.7 | Examination/ Observation | See table A7_5_3_1_2-3 | |
| 3.4.8 | Statistics | Body weight and feed consumption:<br>The control group mean data was compared using t-test with $P \leq 0.05$ (Sokal, R.R. & F.J. Rohlf (1969): Biometry. Freeman & Co, San Fransisco, USA) and all body weight gain data of treatment group was subjected to analysis of variance (ANOVA) with $P \leq 0.05$ (Sokal, R.R. & F.J. Rohlf (1969)). If ANOVA indicated significant treatment effects, the means of the treatment levels were compared to that of controls using the Williams test (Williams, D.A.: A test for differences between treatment means when several dose levels are compared with a zero dose control. Biometrics, 27, 103-117. Williams, D.A.: The comparison of several dose levels with a zero dose control. Biometrics, 28, 519-531). When a treatment mean was significantly different from the control means, that treatment was considered a toxicant effect level. All statistical analysis were conducted using software supplied by SAS Institute Inc., Cary, North Carolina, USA. | |
| | | **4 RESULTS** | |
| **4.1** | **Limit Test / Range finding test** | Limit test was performed | |
| 4.1.1 | Concentration | See data given below | |
| 4.1.2 | Number/ percentage of animals showing adverse effects | See data given below | |
| 4.1.3 | Nature of adverse effects | See data given below | |
| **4.2** | **Results test substance** | | |
| 4.2.1 | Applied concentrations | Nominal concentration in diet: 5000 ppm;<br>measured concentrations: see table A7_5_3_1_2-3 | X |
| 4.2.2 | Effect data (Mortality) | One mortality was noted in the treatment group on day 5. Gross clinical observations (i.e. face was pecked) and postmortem examination of this | |

| | | |
|---|---|---|
| | | bird suggest that death was due to cage mate aggression. No deaths which could be directly attributed to test substance occurred. |
| 4.2.3 | Body weight | See table A7_5_3_1_2-5 |
| 4.2.4 | Feed consumption | See table A7_5_3_1_2-5 |
| 4.2.5 | Concentration / response curve | Not applicable |
| 4.2.6 | Other effects | No clinically observable signs of toxicity were noted in treated birds.<br>No compound-related gross lesions were noted in postmortem examination of birds sacrificed at study termination.<br>Not test substance-related effects: hock and nares scabs (1 bird), prominent keel bone (1 bird), empty crop (1 bird), postmortem autolysis (1 bird) |
| **4.3** | **Results of controls** | See table A7_5_3_1_2-5 |
| 4.3.1 | Number/ percentage of animals showing adverse effects | 1 bird showed red renal zone |
| 4.3.2 | Nature of adverse effects | 1 bird showed red renal zone |
| **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 subacute avian dietary toxicity test was conducted to estimate the toxicity of Preventol A4-S to Bobwhite quail (*Colinus virginianus*) when exposed to the diet for a period of 5 days. The test complies with US-EPA FIFRA Guideline, Section 163.71-2 (1984) as well as those of the US-EPA Toxic Substances Control Act (TSCA) and the ASTM Standard Practice (E857-81) "Standard Practice for Conducting Subacute Dietary Toxicity Tests with Avian Species".<br>One group of 10 birds was fed a diet containing 5000 ppm Preventol A4-S for a period of 5 days. Two additional non-treated groups of 10 birds each were maintained as concomitant controls. All groups were maintained on Preventol A4-S free feed for a three-day observation period following the five-day exposure period. |
| **5.2** | **Results and discussion** | One death was observed in the Preventol A4-S exposure group; however, clinically observable signs and necropsy findings indicate that death was due to wounds received as a result of cage mate aggression. No grossly observable signs of toxicity were noted. Statistically significant decreases in body weight occurred in Preventol A4-S treated birds when compared with controls; however, no differences in feed consumption were apparent. No compound-related gross lesions were noted at necropsy of quails sacrificed at study termination. |

## Section A7.5.3.1.2 Short-term toxicity on birds (1)

**Annex Point IIIA XIII 1.2**

| | | |
|---|---|---|
| 5.2.1 | $LC_{50}$ | > 5000 ppm test substance |
| 5.2.2 | NOEC | < 5000 ppm test substance |
| **5.3** | **Conclusion** | Two of the three validity criteria for short-term avian toxicity test according to OECD Guideline 205 are fulfilled:<br>1. The mortality rate in the control was below 10%,<br>2. Test substance concentration is > 80% of nominal concentration throughout the dosing period.<br>One criterion is not fulfilled: the lowest treatment level causing no compound-related mortality or other observable toxic effects. |
| 5.3.1 | Reliability | 1 |
| 5.3.2 | Deficiencies | No |

## Section A7.5.3.1.2 Short-term toxicity on birds (1)

**Annex Point IIIA XIII 1.2**

| Evaluation by Competent Authorities | |
|---|---|
| | Use separate "evaluation boxes" to provide transparency as to the comments and views submitted |
| | **EVALUATION BY RAPPORTEUR MEMBER STATE** |
| **Date** | 13/12/04 |
| **Materials and Methods** | [redacted] |
| **Results and discussion** | [redacted] |
| **Conclusion** | |
| **Reliability** | |
| **Acceptability** | |
| **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.*<br>*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_5_3_1_2-1: Method of administration of the test substance**

| Carrier/Vehicle | Details |
|---|---|
| Water | No |
| Organic carrier | Yes, corn oil and ethanol |
| Concentration of the carrier [% v/v] | Diet preparation: Appropriate amounts of Preventol A4-S, corn oil and ethanol were combined in a 250 ml Erlenmeyer flask and added to the feed while mixing in a Hobart mixer.<br>Feed for control groups: 0 ppm Preventol A4-S, 160 g corn oil, 150 ml ethanol, 15.84 kg feed.<br>Feed for treatment group: Control: 5000 ppm Preventol A4-S (= 39.548 mg), 70 g corn oil, 150 ml ethanol, 6.89 kg feed. |
| Other vehicle | Yes, feed (Purina Gamebird Startena) |
| Function of the carrier / vehicle | Facilitation of uptake |

**Table A7_5_3_1_2-2: Test animals**

| Criteria | Details |
|---|---|
| Species/Strain | Bobwhite quail (*Colinus virginianus*) |
| Source | [redacted] |
| Age (in weeks), sex and initial body weight (bw) | At an age of 3 days the quails were obtained;<br>Sex: unknown;<br>Body weights at age of 10 days: 18-21 g |
| Breeding population | No data |
| Amount of food | Food and water were available ad libitum, prior to and throughout the study. |
| Age at time of first dosing | Age: 10 days, body weights 18-21 g |
| Health condition / medication | No prophylactic medication. |

**Table A7_5_3_1_2-3: Test system**

| Criteria | Details |
|---|---|
| Test location | Indoor, in steel brooders |
| Holding pens | galvanized steel brooders (90 x 70 x 23 cm), pelletized wood was used as cage bedding und was changed once during the study. |
| Number of animals | 30 (unknown sex) |
| Number of animals per pen [cm²/bird] | 10 birds of unknown sex (630 cm²/bird) |
| Number of animals per dose | Two control groups, each 10 birds of unkown sex, One dose group with 10 birds of unkown sex |
| Pre-treatment / acclimatisation | Acclimatisation period 7 days, birds were examined upon receipt and daily throughout the acclimatisation period. Less than 5% mortality was noted prior test initiation and all unsuitable birds (injured, deformed etc.) were eliminated from inclusion in the test. Food (Purina Gamebird Startena) and water were available ad libitum, prior to and throughout the study. |
| Diet during test | Food (Purina Gamebird Startena) and water were available ad libitum throughout the study. |
| Dosage levels (of test substance) | Nominal concentration in diet: 5000 ppm; the birds were fed for 5 days;<br>measured concentrations: 4892 ± 73 ppm (day 0, three samples taken for homogeneity analysis), 5570 ppm (sample taken on day 5 from initial feed mix for stability determination). |
| Replicate/dosage level | One dose group with 10 birds; gang housed in a breeder |
| Feed dosing method | Orally by feed |
| Dosing volume per application | The group of birds was fed a diet containing 5000 ppm a.i. for a period of 5 days, ad libitum. |
| Frequency, duration and method of animal monitoring after dosing | After 5 treatment days, birds were given control feed for 3 days (post-exposure observation period). Observations for mortality and toxic signs were made twice daily except on weekends when only one observation per day was made; feed consumption for each group was recorded daily. At the end of the study, all surviving birds were sacrificed by $CO_2$ asphyxiation. Necropsy examinations were conducted on all birds at study termination, as well as on all birds that died during the in-life phase of the study. |
| Time and intervals of body weight determination | Body weights were recorded on day 0, 5 and 8 |

**Table A7_5_3_1_2-4: Test conditions (housing)**

| Criteria | Details |
|---|---|
| Test temperature | 37.8 ± 0.5 °C. |
| Shielding of the animals | No data |
| Ventilation | No data |
| Relative huminity | No data |
| Photoperiod and lighting | 8/16 hour light/dark cycle |

**Table A7_5_3_1_2-5: Average body weight change and feed consumption of animals during study**

| | | | CONTROL 1 | CONTROL 2 | DOSE GROUP |
|---|---|---|---|---|---|
| Mean body weight [g] | Treatment days | Day 0 | 17.5 | 16.9 | 18.3 |
| | | Day 5 | 30.2 | 29.6 | 25.6 |
| | Observation day | Day 8 | 38.6 | 38.6 | 33.9 |
| Daily food consumption [g/bird/day] | Treatment days | Day 1 | 5.5 | | 4.0 |
| | | Day 2 | 4.9 | | 3.7 |
| | | Day 3 | 3.4 | | 3.3 |
| | | Day 4 | 5.2 | | 6.8 |
| | | Day 5 | 6.5 | | 6.8 |
| | Observation days | Day 6 | 6.0 | | 6.5 |
| | | Day 7 | 5.3 | | 4.7 |
| | | Day 8 | 4.2 | | 3.6 |
| | | Mean | 5.1 | | 4.9 |

**Table A7_5_3_1_2-6: Validity criteria for short-term avian toxicity test according to OECD Guideline 205**

| | fulfilled | Not fulfilled |
|---|---|---|
| Mortality of control animals < 10% | X | |
| Test substance concentration > 80% of nominal concentration throughout the dosing period | X | |
| Lowest treatment level causing no compound-related mortality or other observable toxic effects | | X |

## Section A8

**Annex Point IIA VIII8.1-8.6 & IIIA VIII.1**

**MEASURES NECESSARY TO PROTECT MAN, ANIMALS AND THE ENVIRONMENT**

| | | Official use only |
|---|---|---|
| **REFERENCE** | Bayer Chemicals, 2003, Preventol A 4 S, Safety Data sheet, Bayer Chemicals, SDS No. 014730/28, 2003-10-01 | |
| **8.1**<br>**Recommended methods and precautions concerning handling, use, storage, transport or fire** | Handling, use and storage:<br>Dichlofluanid is harmful by inhalation and irritating to eyes. It may cause sensitisation by skin contact. Therefore suitable protective clothing, including protective gloves (e.g. of rubber, Polyvinyl chloride – PVC), closely fitting goggles and in case of dust formation respiratory protection with particle filter, e.g. DIN 3181 P 2 must be worn.<br><br>Recommended container materials for the direct contact with the active substance: Polypropylene plastic material (PP), high and low density polyethylene plastic materials (HDPE, LDPE).<br><br>VCI storage class: 11<br><br>Transport:<br>GGVSee/IMDG Code: 9 UN No. 3077<br>EmS: NO<br>PG: III MPO: NO<br>GGVSE: Class 9 RID/ADR: Class 9<br>Warning sign: Hazard no. 090 Substance no. 3077<br>ADNR: Class 9<br>Cat -- ICAO/IATA-DGR: 9 3077 III<br><br>Fire:<br>Extinguishing media: water, foam, $CO_2$, dry powder | X |
| **8.2**<br>**In case of fire, nature of reaction products, combustion gases, etc.** | Formation of carbon monoxide, carbon dioxide, hydrogen halides, sulphur dioxide, nitrogen oxides and other toxic gases in the event of fire or during thermal decomposition. | |
| **8.3**<br>**Emergency measures in case of an accident** | Personal precautions:<br>Dichlofluanid irritates eyes, respiratory system and skin. It may cause sensitisation by skin contact.<br>Respiratory protection, protective gloves and closely fitting goggles should be worn when handling this material.<br><br>Environmental precautions:<br>Dichlofluanid and its container must be disposed of as hazardous waste.<br><br>Methods of cleaning Up:<br>Take up spilled product with dust-binding material or suitable vacuum cleaner. Avoid formation of dust. Put materials taken up into labelled, sealable container. To clean the floor and all objects contaminated by this material, use dilute alkalis. | |
| **8.4**<br>**Possibility of destruction or decontamination following release in or on the following: (a) air (b) water, including drinking water (c) soil** | Procedures for the decontamination of water in the event of an accident:<br>As dichlofluanid is hydrolysed very fast in the aquatic environment, only DMSA is regarded as relevant concerning decontamination. As the toxicity of DMSA is low to aquatic organisms (fish, daphnids, green algae), it is assumed that procedures for neutralisation in water would burden natural aquatic environments more than DMSA itself. Therefore, no specific procedures are recommended. | |

## Section A8

**Annex Point IIA VIII8.1-8.6 & IIIA VIII.1**

**MEASURES NECESSARY TO PROTECT MAN, ANIMALS AND THE ENVIRONMENT**

**8.5**

**Procedures for waste management of the active substance for industry or professional users**

8.5.1

Possibility of re-use or recycling

8.5.2

Possibility of neutralisation of effects

8.5.3

Conditions for controlled discharge including leachate qualities on disposal

8.5.4

Conditions for controlled incineration

Detailed instructions for safe disposal:
Examine possibilities for re-utilization. Package product wastes. Close and label the waste receptacles and, likewise, any uncleaned empty containers. Dispose of them at a suitable waste incineration plant in accordance with the official regulations. Where large quantities are concerned, consult the supplier. When uncleaned empty containers are passed on, the recipient must be warned of any possible hazard that may be caused by residues. For disposal within the EC, the appropriate code according to the European Waste Catalogue (EWC) should be used.

Methods other than controlled incineration for disposal of the active substance contaminated packaging and contaminated materials:
Applications that involve controlled incineration with energy recovery are considered to be the most environmentally-acceptable means of disposal. However, where local recovery schemes exist, these should also be considered.

**8.6**

**Observations on undesirable or unintended side-effects, e.g. on beneficial and other non-target organisms**

**8.7**

**Identification of any substances falling within the scope of List I or List II of the Annex to Directive 80/68/EEC on the protection of ground water against pollution caused by certain dangerous substances**

Organohalogen compounds are covered by List I of the Annex to Directive 80/68/EEC.

Biocides and their derivatives are covered by List II of the Annex to Directive 80/68/EEC.

## Section A8

**Annex Point IIA VIII8.1-8.6 & IIIA VIII.1**

**MEASURES NECESSARY TO PROTECT MAN, ANIMALS AND THE ENVIRONMENT**

| | Evaluation by Competent Authorities |
|---|---|
| | Use separate "evaluation boxes" to provide transparency as to the comments and views submitted |
| | **EVALUATION BY RAPPORTEUR MEMBER STATE** |
| **Date** | 16/02/05 |
| **Materials and Methods** | [redacted] |
| **Results and discussion** | [redacted] |
| **Conclusion** | |
| **Reliability** | |
| **Acceptability** | |
| **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.*<br>*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** | |