## Section A7.1.2.2.2 Water/sediment degradation study

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| | | | Official use only |
|---|---|---|---|
| | | **1 REFERENCE** | |
| **1.1** | **Reference** | K. Scholz, 1987, Degradation of Dichlofluanid in Water-Sediment Systems. Bayer AG, Crop Protection Development, Institute for Metabolism Research, PF Report No. 2800, 1987-06-02 | |
| **1.2** | **Data protection** | Yes | |
| 1.2.1 | Data owner | Bayer Crop Science AG | |
| 1.2.2 | Companies with letter of access | Bayer Chemicals AG | |
| 1.2.3 | Criteria for data protection | Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA | |
| | | **2 GUIDELINES AND QUALITY ASSURANCE** | |
| **2.1** | **Guideline study** | No, no guidelines available, methods developed in co-operation with the Dutch authorities, comparable to (later) EPA guidelines | |
| **2.2** | **GLP** | No, GLP was not compulsory at the time the study was performed | |
| **2.3** | **Deviations** | No | |
| | | **3 MATERIALS AND METHODS** | |
| **3.1** | **Test material** | a) [Phenyl-UL-$^{14}$C] dichlofluanid<br>b) non-active standard substance (dichlofluanid) | |
| 3.1.1 | Lot/Batch number | No lot or batch no. mentioned | |
| 3.1.2 | Specification | a) specific radioactivity was 1246.9 kBq/mg, sample provided from Bayer AG, Isotope Laboratory, Dr. Marsmann, Elberfeld, Germany.<br>b) see purity, sample provided by Dr. Krohn (Elberfeld) | |
| 3.1.3 | Purity | a) [redacted] radiochemical purity<br>b) [redacted] purity | |
| 3.1.4 | Further relevant properties | No problems related to abiotic stability or volatility are expected from the data available | |
| 3.1.5 | Composition of Product | - | |
| 3.1.6 | TS inhibitory to micro-organisms | Not to be expected because of the favourable results of the respiration inhibition tests in soil and sewage sludge | |
| 3.1.7 | Specific chemical analysis | Radio thin layer and HPLC analysis (reversed phase) | |
| **3.2** | **Reference substance** | No | |
| 3.2.1 | Initial concentration of reference substance | - | |
| **3.3** | **Testing procedure** | | |
| 3.3.1 | Inoculum / test | Two different aquatic micro ecosystems (500 ml volume) containing a | X |

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| | species | sediment portion. The water/sediment samples were taken from a fish pond (Lienden, NL) and from a drainage ditch in a fruit orchard (Ijzendoorn, NL). The characterisation of the sediments is shown in table A7_1_2_2_2-1. | |
| 3.3.2 | Test system | see table A7_1_2_2_2-2 | X |
| 3.3.3 | Test conditions | see table A7_1_2_2_2-2 | |
| 3.3.4 | Method of preparation of test solution | Solution I: The radioactive compound (5.93 mg a.i.) was dissolved in 600 µl methanol and the radioactivity measured by liquid scintillation (= 7,394 kBq).<br>Solution II: 19.07 mg of unlabelled a.i. were dissolved in 1400 µl methanol.<br>Solution III = Solution I + solution II = 2000 µl<br><br>Experiment I: 100 µl of solution III was pipetted into each vessel, whereas in experiment II an aqueous suspension of the a.i. was indroduced into the vessels. Experiments 2 and 3 served as controls (exp. 2: influence of solvent; exp. 3: blank control, influence of a.i.). | |
| 3.3.5 | Initial TS concentration | The amount of dichlofluanid applied to the water sediment systems was 2.50 mg/l, which is related to the maximum application rate in agriculture (assumption: dosage is dissolved in a 10 cm deep water area). This quantity was in the region of the water solubility of the a.i. | X |
| 3.3.6 | Duration of test | up to 120 days | |
| 3.3.7 | Analytical parameter (methods) | Radioactivity measurement of volatile compounds:<br>a) sorption on oil coated quartz wool plugs, extraction with ethyl acetate, which was measured by liquid scintillation.<br>b) sorption on sodium carbonate and release of $CO_2$ (after acidification) in a scintillation cocktail.<br>Radioactivity measurement of solid samples (e.g. sediment):<br>pre-treatment by e.g. drying and milling, than combustion and analysing radiolabelled $CO_2$<br><br>The TLC separations were performed on silica gel F254 TLC plates with different solvent systems.<br><br>Methods for visualize the substance spots: 1. extinction of UV-induced fluorescence of the TLC plates; 2. Linear analyser with evaluation unit; 3. autoradiography with x-ray film | |
| 3.3.8 | Sampling | a) Experiment I: processing dates for the incubation vessels were 7, 14, 30, 60 and 120 days after addition of a.i.<br><br>b) Experiment II and III: the two vessels were worked up at the end of the experiment (120 d) | X |
| 3.3.9 | Intermediates/ degradation products | Spectroscopic analysis of the test substance, DMSA and KUE 8630 B: GC-MS (HP 5970 GC 5880 A (DMSA) or HP 1084 B (KUE 8630 B)) | |
| 3.3.10 | Nitrate/nitrite measurement | n.a. | |
| 3.3.11 | Controls | Experiments 2 and 3 served as controls (exp. 2: influence of solvent; exp. 3: influence of a.i.). | X |
| 3.3.12 | Statistics | - | |

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| | | **4 RESULTS** | |
| **4.1** | **Degradation of test substance** | | |
| 4.1.1 | Graph | Degradation curves are provided in the report | |
| 4.1.2 | Degradation | See tables A7_1_2_2_2-3 and A7_1_2_2_2-4 | X |
| 4.1.3 | Other observations | - | |
| 4.1.4 | Degradation of TS in abiotic control | Not relevant, because no hydrolytic degradation can be expected from the data, light induced degradation was excluded by running the experiment in the dark. | X |
| 4.1.5 | Degradation of reference substance | n.a. | |
| 4.1.6 | Intermediates/ degradation products | Main metabolite: DMSA (dimethylaminosulfanilide); no further metabolite exceeded the 10% mark,<br>second metabolite: KUE 8630 B (methylaminosulfanilide) max. 6.9% | X |
| | | **5 APPLICANT'S SUMMARY AND CONCLUSION** | |
| **5.1** | **Materials and methods** | The degradation and metabolism behaviour of [phenyl-UL-$^{14}$C]dichlofluanid in an aquatic micro ecosystem containing a sediment portion was investigated in two different water/sediment systems. The water/sediment samples were taken from a fish pond (Lienden, NL) and from a drainage ditch in a fruit orchard (Ijzendoorn, NL). A test system was used which was developed with the Dutch authorities. Material balances were performed using by radioactivity measurements of all test components. | |
| **5.2** | **Results and discussion** | Dichlofluanid was so rapidly degraded to the metabolite dimethylaminosulfanilide (DMSA) by the water-sediment systems that already after 7 days the active ingredient was no longer detectable. Besides DMSA another metabolite, methylaminosulfanilide (KUE 8630 B), occured at levels of up to 6.9%. After 120 days 3.3% (Ijzendoorn) and 5.1% (Lienden) of the applied radioactivity was detectable as [14C]$CO_2$. 69.2% (Ijzendoorn) and 63.5% (Lienden), respectively, of the applied radioactivity was in the water while 25.8% (Ijzendoorn) and 23.9% (Lienden), respectively, had moved to the sediment during the incubation time of 120 days. 17.6% (Ijzendoorn) and 18.7% (Lienden), respectively, could not be extracted from the sediment using the described extraction procedure. | X |
| **5.3** | **Conclusion** | The results in this test show that dichlofluanid was very rapidly degraded in aerobic aquatic systems to DMSA (dimethylaminosulfanilide). There was no further metabolite approaching or exceeding the 10% mark within the incubation time. Dichlofluanid does not constitute a lasting potential to contaminate surface water or sediment.<br><br>The study is well documented and reported. A complete material balance was performed at all samplings by radioactive analysis. | X |
| 5.3.1 | Reliability | Reliability indicator: 2 | |

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| 5.3.2 | Deficiencies | Yes,<br>No lot or batch no. of test compound mentioned |

| Evaluation by Competent Authorities | |
|---|---|
| | Use separate "evaluation boxes" to provide transparency as to the comments and views submitted |
| | **EVALUATION BY RAPPORTEUR MEMBER STATE** |
| **Date** | 30/11/2004 |
| **Materials and Methods** | Applicant's version is acceptable with the following comments:<br>**3.3.1** The characterisation of the sediments is based on a mixture of samples taken from 1985 and 1987.<br>**3.3.2** The preparation of water-sediment samples is not clear - the study report states the sediment component was not resuspended but the water-sediment systems were filtered and 50g dry weight of sediment used in each water-sediment system.<br>**3.3.5** The initial test substance concentration was 2.5 mg/l (related to maximum application rate in agriculture), but it is unclear whether dichlofluanid was fully soluble at this concentration.<br>**3.3.8** There were no processing dates for the incubation vessels below 7 days, by which time no dichlofluanid remained.<br>**3.3.11** Although controls were used to monitor the influence of solvent and active ingredient, no abiotic control was included. |

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| **Results and discussion** | Applicant's version is acceptable with the following comments:<br>**4.1.2** Dichlofluanid was rapidly degraded such that after 7 days no dichlofluanid remained. The lack of analysis at time points below 7 days has prevented the degradation half-life being determined.<br><br>**4.1.2 and 5.2** There is a slight discrepancy in the radioactivity data reported for sediment from Lienden after 120 days. From table A7_1_2_2_2-3 the sediment (non-extractable) is 19.0 % whereas in the text in section 5.2 it is stated as 18.7 %.<br><br>**4.1.4** The statement that no hydrolytic degradation is expected seems unrealistic, as hydrolysis is likely with the pH around 8. Resultant degradation could be a mixture of hydrolysis and biodegradation, as no abiotic control was included in the study. Photolysis was excluded by carrying out the experiment in the dark.<br><br>**4.1.6** The initial degradation product formed was DMSA, which occured in both the supernatant and sediment phases (distribution roughly 7:1). DMSA appeared to slowly degrade, with the formation of methylaminosulfanilide, MSA (up to 4.1 - 6.5 % in the supernatant and up to 0.4 % extractable from the sediment) and an unidentified component (up to 1.9 - 3.4 % in the supernatant) after 120 days. A small amount of carbon dioxide was also detected after 120 d in the trap and supernatant (about 3.3 - 5.1 %). Some overall loss of radioactivity from the supernatant was observed during the experiment, along with an increase in the amount of radioactivity associated with an unextracted component on the sediment. After 120 d the distribution of radioactivity was 64 – 69 % in the supernatant phase, 24 – 26 % extractable from the sediment and about 18 – 19 % remaining unextracted on the sediment after methanol:water and methanol extractions. |
| **Conclusion** | Applicant's version is acceptable with the following comments:<br><br>**5.2** and **4.1.2** Details described under 4.1.2 above.<br><br>**5.3** The degradation of dichlofluanid was likely to be a mixture of hydrolysis and biodegradation, but without an abiotic control the importance of the specific mechanisms could not be distinguished. A simple mechanism for the breakdown of dichlofluanid to DMSA, and the latter to MSA was proposed. However, over the length of the experiment the radioactivity in the supernatant decreased whilst that associated with an unextracted component on the sediment increased to about 18 - 19 %.<br><br>5.4 The two water-sediment systems (fish pond and drainage ditch) had different properties especially regarding particle sizes, nitrogen, phosphorus and organic carbon. However the data obtained from both systems were similar suggesting that the results seen could be more widely applicable. |
| **Reliability** | 2 |
| **Acceptability** | Acceptable |
| **Remarks** | All endpoints and data presented in the summary and tables have been checked against the original study and are correct. |
| | **COMMENTS FROM ...** |

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| **Date** | *Give date of comments submitted* |
| **Materials and Methods** | *Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.*<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_1_2_2_2-1: Properties of the Natural Water Sediment Systems**

| | Particle Size Distribution | | | N(total) | P(total) | C org. | pH | $CaCO_3$ |
|---|---|---|---|---|---|---|---|---|
| System | 16-2000 µm [%] | 16 µm [%] | 2 µm [%] | [g/100 g] | [g/100 g] | [%] | | [%] |
| IJzendoorn | 50 | 43 | 25 | 0.32 | 0.23 | 4.6 | 8.4 | 2.6 |
| Lienden | 88 | 9 | 5 | 0.02 | 0.05 | 0.5 | 8.5 | 2.5 |

**Table A7_1_2_2_2-2: Test system and Test conditions**

| **Criteria** | **Details** |
|---|---|
| Culturing apparatus | Glass vessels containing 500 ml water and 10% (w/w) sediment (portions corresponding to 50 g dry weight), a system to adsorb $CO_2$ and other volatile substances. |
| Number of culture flasks/concentration | 8 flasks for each of the two water sediment system,<br>1 flask served as control for the influence of solvent,<br>1 flask was blank system (control for influence of a.i.) |
| Aeration device | Not applied |
| Measuring equipment | Measurements of pH, oxygen content, redox potential and temperature of the water were performed from each sampling vessel and in addition from the two control vessels. |
| Composition of medium | see table A7_1_2_2_2-1 |
| Additional substrate | No |
| Pre-incubation of the test systems | yes, 14 days |
| Test temperature | 22 ± 1 °C |
| pH | Ijzendoorn: 7.8-8.2 (blank (8.0-8.5),<br>Lienden: 8.1-8.4 (blank 7.9-8.8) |
| Oxygen content (in % of maximum oxygen content: at 22°C: 8.73 mg $O_2$/l) | Ijzendoorn: 87-93 (blank (91-98),<br>Lienden: 86-93 (blank 93-99) |
| TOC content at the beginning of the study | Ijzendoorn: 3 mg/l,<br>Lienden: 13 mg/l |
| Aeration of dilution water | No |
| Suspended solids concentration | not determined |
| Other relevant criteria | a) the test was conducted in the dark,<br>b) the water phase was slowly stirred by a magnetic stirrer to maintain oxygen uptake |

**Table A7_1_2_2_2-3: Distribution of radioactivity [% of applied] in two water/sediment systems after application of 2.50 mg/l [phenyl-UL-$^{14}$C]dichlofluanid**

| | Ijzendoorn | | | | | Lienden | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | days incubation | | | | | days incubation | | | | |
| | 7 | 14 | 30 | 60 | 120 | 7 | 14 | 30 | 60 | 120 |
| **supernatant water** | **87.1** | **87.4** | **81.8** | **75.8** | **69.2** | **87.6** | **84.5** | **82.3** | **73.3** | **63.5** |
| sediment extractable | 10.9 | 11.3 | 12.0 | 10.9 | 8.2 | 9.0 | 8.1 | 7.9 | 6.6 | 4.9 |
| sediment non extractable | 1.7 | 2.0 | 5.8 | 8.3 | 17.6 | 2.1 | 3.2 | 5.8 | 9.7 | 19.0 |
| **sediment** | **12.6** | **13.3** | **17.8** | **19.2** | **25.8** | **11.1** | **11.3** | **13.7** | **16.3** | **23.9** |

**Table A7_1_2_2_2-4: Distribution of dichlofluanid and metabolites [% of applied radioactivity] in two water/sediment systems after application of 2.50 mg/l [phenyl-UL-$^{14}$C]dichlofluanid**

| | | Ijzendoorn | | | | | Lienden | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | days incubation | | | | | days incubation | | | | |
| | | 7 | 14 | 30 | 60 | 120 | 7 | 14 | 30 | 60 | 120 |
| **water** | **total** | **87.1** | **87.4** | **81.8** | **75.8** | **69.2** | **87.6** | **84.5** | **82.3** | **73.3** | **63.5** |
| | Dichlofluanid | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| | DMSA | 87.1 | 87.4 | 81.8 | 73.2 | 63.2 | 87.6 | 84.5 | 82.3 | 66.5 | 53.6 |
| | KUE 8630 B | < 0.1 | < 0.1 | < 0.1 | 2.6 | 4.1 | < 0.1 | < 0.1 | < 0.1 | 3.7 | 6.5 |
| | unidentified | < 0.1 | < 0.1 | < 0.1 | < 0.1 | 1.9 | < 0.1 | < 0.1 | < 0.1 | 3.1 | 3.4 |
| **sediment** | **total** | **10.9** | **11.3** | **12.0** | **10.9** | **8.2** | **9.0** | **8.1** | **7.9** | **6.6** | **4.9** |
| | Dichlofluanid | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| | DMSA | 10.9 | 10.9 | 11.6 | 10.5 | 7.7 | 9.0 | 7.8 | 7.6 | 6.3 | 4.4 |
| | KUE 8630 B | < 0.1 | 0.1 | 0.2 | 0.3 | 0.4 | < 0.1 | 0.1 | 0.2 | 0.2 | 0.4 |
| | unidentified | < 0.1 | 0.3 | 0.3 | 0.1 | 0.1 | < 0.1 | 0.2 | 0.1 | 0.1 | 0.1 |