Section A7.1.2.2.2 Water/sediment degradation study

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Official use only

		1 REFERENCE
1.1	**Reference**	K. Scholz, 1997, Aerobic Degradation of Dichlofluanid in Water-Sediment. Bayer AG, Crop Protection Development, Institute for Metabolism Research and Residue Analysis, Report No. MR-948/97 (PF Report No. 4319), 1997-12-10
1.2	**Data protection**	Yes
1.2.1	Data owner	Bayer Crop Science AG
1.2.2	Companies with letter of access	Bayer Chemicals AG
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, German BBA Guideline Part IV, 5-1 (December 1990)
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	a) [Phenyl-UL-^{14}C] dichlofluanid b) non-active standard substance (dichlofluanid)
3.1.1	Lot/Batch number	a) [Phenyl-UL-^{14}C] dichlofluanid: Batch 31/1 b) non-active standard substance (dichlofluanid): Batch: [redacted]
3.1.2	Specification	a) specific radioactivity was 3.76 MBq/mg, sample provided from Bayer AG, Isotope Laboratory, Elberfeld, Germany. b) see purity, sample provided by Dr. Krohn (Leverkusen)
3.1.3	Purity	a) [redacted] radiochemical purity 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	a) radiochemical purity: HPLC, radioactivity detector and TLC, scan b) chemical purity: HPLC, UV detector
3.2	**Reference substance**	No
3.2.1	Initial concentration of reference substance	-

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3.3	**Testing procedure**		
3.3.1	Inoculum / test species	The water/sediment samples were taken from an artificially dammed pond (Hönniger Weiher, Wipperfürth, Germany) and a fenced-in fishing pond (Angler Weiher, Leverkusen, Germany)	
3.3.2	Test system	see table A7_1_2_2_2-2 In order to determine the exact DT-50 values two experiments were performed: a) Experiment I: performed only with the supernatant water (in 1 litre Erlenmeyer flask) b) Experiment II: carried out with water and sediment to confirm the results obtained with supernatant water only (500 ml microecosystem)	
3.3.3	Test conditions	see table A7_1_2_2_2-2	
3.3.4	Method of preparation of test solution	a) Experiment I: the test substance used was pure radio-labelled dichlofluanid. The radioactive compound was dissolved in 4.5 ml acetonitrile (Application solution I) and the radioactivity measured by liquid scintillation. A total of 450 µl (= 1,148.940 kBq) of Application solution I was applied to the vessels (= 0.306 mg a.i./500 ml water). b) Experiment II: the test substance used was a mixture of radio-labelled and unlabelled dichlofluanid. A total of 86 µl (11.2 mg dichlofluanid diluted in 1120 µl acetonitrile) was pipetted into a vessel and the solvent was evaporated. Application solution I (2500 µl) was added and radioactivity was determined (Application solution II). 300 µl (= 771.261 kBq) of Application solution II was applied to the vessels (= 0.308 mg/500 ml water + sediment)	X
3.3.5	Initial TS concentration	The amount of dichlofluanid applied to the water sediment systems was 0.60 mg/l. The maximum application rate in agriculture is up to 2.5 kg/ha, this amount corresponds to 0.83 mg/l (based on water depth of 30 cm). Since 0.83 mg/l is higher than 50% of the water solubility of dichlofluanid, this concentration was not used.	
3.3.6	Duration of test	up to 7 days	
3.3.7	Analytical parameter (methods)	Thin-Layer Chromatography: silica gel plates and RP-18 plates with different solvents methods for visualisation: autoradiography (radiolabelled compounds), UV lamp (unlabelled compounds). Spectroscopic analysis of the test substance and DMSA: GC-MS (INCOS XL instrument by Finnigan with Varian gas chromatograph) Radioactivity measurement of volatile compounds: a) Sorption on polyurethane foam plugs, extraction with ethyl acetate, which was measured by liquid scintillation. b) Sorption on sodium carbonate and release of CO_2 (after acidification) in a scintillation cocktail. Radioactivity measurement of solid samples (e.g. sediment): pre-treatment by e.g. drying and milling, then combustion and analysing radiolabelled CO_2	
3.3.8	Sampling	a) Experiment I: processing dates for the incubation vessels were 0.5 h, 2 h, 4 h, 7 h, 12 h, 17 h, 24 h, 3 days and 7 days	

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		b) Experiment II: processing dates for the incubation vessels were 1.5 h, 3.5 h, 4 h and 7 h	
3.3.9	Intermediates/ degradation products	Spectroscopic analysis of the test substance: GC-MS (INCOS XL instrument by Finnigan with Varian gas chromatograph)	
3.3.10	Nitrate/nitrite measurement	n.a.	
3.3.11	Controls	no control vessels	
3.3.12	Statistics	-	
		4 RESULTS	
4.1	**Degradation of test substance**		
4.1.1	Graph	Provided in the report	
4.1.2	Degradation	See tables A7_1_2_2_2-3 and A7_1_2_2_2-4	
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	DMSA (dimethylaminosulfanilide); no further metabolite exceeded the 10% mark, DMSA degraded to CO_2	X
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The degradation and metabolism behaviour of [phenyl-UL-^{14}C]dichlofluanid was investigated in two experiments. With experiment I the degradation of dichlofluanid was investigated in two aquatic model ecosystems consisting of surface water only; experiment II was performed with water and sediment portion. Two different water/sediment systems were investigated according to BBA guideline IV, 5-1 (December 1990). Material balances were performed using radioactivity measurements of all test components.	
5.2	**Results and discussion**	The calculated DT-50 values (disappearance time of 50%) for dichlofluanid in the supernatant water of the two water-sediment systems were 1.1 and 2.7 hours. These values are relevant for natural surface water bodies. The DT-50 values for the total system of water and sediment were 1.2 and 3.0 hours (Experiment II) and for the supernatant water without sediment 1.5 and 3.0 hours (Experiment I). Dichlofluanid was as fast degraded in water-sediment systems as in systems consisting of water only.	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.	X

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		Dichlofluanid does not constitute a lasting potential to contaminate surface water or sediment. The study is well documented and reported. A complete material balance was performed at all samplings by radioactive analysis. The parameters from two blank water sediment systems show no deviations from the fortified systems.
5.3.1	Reliability	Reliability indicator: 1
5.3.2	Deficiencies	No

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 comment: **3.3.4** The stability of the dichlofluanid in acetonitrile stock solution used to spike the test samples was confirmed before and after the spiking, although the chromatographic data provided in the report were insufficient to substantiate this conclusion.
Results and discussion	Applicant's version is acceptable with the following comments: **4.1.4** The statement that no hydrolytic degradation is expected seems unrealistic, as hydrolysis has been shown to occur at the reported pH values. **4.1.6** The comments that DMSA is the only degradation product at > 10 %, and that it degraded to carbon dioxide are somewhat misleading. It is likely that DMSA is the only significant breakdown product seen because of the short time of the test, whilst the degradation of DMSA to carbon dioxide may occur but was not supported by any test data obtained.
Conclusion	Applicant's version is acceptable with the following comments: **5.2** The calculated half-life for dichlofluanid in the supernatant water of water-sediment systems (1.1 - 2.7 hours) appears shorter than when in 'pure' water (18.8 hours), suggesting that both hydrolysis and biodegradation can occur and that the shorter half-lives are more realistic for use in environmental risk assessment. The correlation between the half-life and water-sediment system characteristics eg organic carbon may need to be considered. **5.3** The limited time-scale of this study means that insufficient information is generated on the ultimate fate of the dichlofluanid, and hence extrapolations regarding its breakdown mechanism should be avoided.
Reliability	1
Acceptability	Acceptable
Remarks	All endpoints and data presented in the summary and tables have been checked against the original study and are correct.

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	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_1_2_2_2-1: Properties of the Natural Water Sediment Systems

System	Property	Hönniger Weiher	Angler Weiher
Supernatant water	Hardness [dH°]	4.1	12.1
	N(total) [mg/l]	2.0	2.4
	P(total) [mg/l]	0.6	0.5
	TOC	1.6	1.7
	DOC	1.6	1.7
Sediment	Sediment (0-10 cm)	loam	sandy loam
	Texture analysis (USDA); sand/silt clay [%]	38.5/47.1/14.4	69.0/21.8/9.2
	pH (in water/in 0,01 M CaCl2)	5.8/5.4	7.3/6.7
	CEC [meq/100 g dry sediment]	10	< 1
	Organic carbon/humus [mg/100 g dry sediment]	4070/7000	2310/3970
	N(total) [mg/100 g dry sediment]	310	180
	P(total) [mg/100 g dry sediment]	89.4	37.4

Table A7_1_2_2_2-2: Test system and Test conditions

Criteria	Details
Culturing apparatus	a) Experiment I: 500 ml samples of supernatant water were pored into 1 litre Erlenmeyer flasks); b) Experiment II: carried out with water and sediment in microecosystems; the glass vessels containing 310 ml water and 190 ml sediment (to reach a sediment height of 2.5 cm); total volume: 500 ml each. Dry weight of sediment in flask: 128.6/163.0 g (Hönniger Weiher/Angler Weiher)
Number of culture flasks/concentration	a) Experiment I: total of 4 batches (two water systems, each replicates A and B) b) Experiment II: total of 6 batches (two water systems, partly two replicates A and B)
Aeration device	Not applied
Measuring equipment	In the supernatant water measurements of the oxygen content, pH-value and redox potential were performed; the redox potential of the sediment was also determined during the experiments.
Composition of medium	see table A7_1_2_2_2_1
Additional substrate	No
Pre-incubation of the test systems	yes, 22 days
Test temperature	20.5 ± 0.5 °C
pH at the begin/end of the study	Experiment I: Hönniger Weiher: 7.4/7.7, Angler Weiher: 8.0/8.1 Experiment II: Hönniger Weiher: 7.5/7.6, Angler Weiher: 8.1/8.1
Oxygen content at the begin of the study (in % of maximum oxygen content: at 20°C: 8.84 mg O_2/l)	Experiment I: Hönniger Weiher: 94/90%, Angler Weiher: 86/90% Experiment II: Hönniger Weiher: 95/91%, Angler Weiher: 88/88%
Aeration of dilution water	No
Suspended solids concentration	not determined
Other relevant criteria	a) the test was conducted in the dark, b) the water phase was slowly stirred by a magnetic stirrer to maintain oxygen uptake

Table A7_1_2_2_2-3: Distribution of dichlofluanid and DMSA [% of applied radioactivity] in natural water after application of 0.60 mg/l [phenyl-UL-^{14}C]dichlofluanid (Experiment I)

	Incubation time						
	0 min	0.5 h	2 h	7 h	1 d	3 d	7 d
Hönniger Weiher							
water after extraction	0.1	0.1-0.2	0.1-0.2	0.3	0.3	0.3	0.4-0.5
Dichlofluanid (dichloromethan extr.)	99.4	90.8	67.3-70.6	14.8-16.3	< 0.1	< 0.1	n.d.
DMSA (dichloromethan extr.)	0.5	7.9-9.1	27.4-31.5	79.9-84.3	98.3-98.5	99.2-100	97.6-98.3
Unknown(s)	n.d.	n.d.	n.d.	n.d.	0.1	n.d.	n.d.
Angler Weiher							
water after extraction	0.1	0.1-0.2	0.2-0.3	0.3-0.4	0.3-0.4	0.3	0.3-0.4
Dichlofluanid (dichloromethan extr.)	99.2	79.2-80.7	41.6-42.5	1.8-2.1	< 0.1	< 0.1	n.d.
DMSA (dichloromethan extr.)	0.7	19.1	56.2-56.9	96.9-98.5	96.9-97.0	99.2-99.7	98.2-99.3
Unknown(s)	n.d.	n.d.	n.d.	n.d.	0.1-< 0.1	n.d.	n.d.

Table A7_1_2_2_2-4: Distribution of radioactivity [% of applied] in two water/sediment systems after application of 0.60 mg/l [phenyl-UL-^{14}C]dichlofluanid (Experiment II)

		Hönniger Weiher incubation time			Angler Weiher incubation time		
		0 min	3.5 h	7 h	0 min	1.5 h	4 h
supernatant water	**total**	**100.0**	**79.9-82.8**	**83.8**	**100.0**	**81.5-83.4**	**83.7**
	water after extraction	0.1	0.8	1.3	0.1	1.1-1.3	1.1
	Dichlofluanid (dichloromethan extract)	99.4	43.5-50.7	2.2	99.2	30.9-44.9	9.1
	DMSA (dichloromethan extract)	0.5	28.4-38.5	80.3	0.7	35.5-51.2	73.4
sediment	**total**	**0.0**	**13.7-14.3**	**13.0**	**0.0**	**12.2-13.9**	**12.0**
	Dichlofluanid (organic sediment extract)	0.0	5.7-6.9	2.1	0.0	4.0-6.9	0.3
	DMSA (organic sediment extract)	0.0	7.1-7.7	10.6	0.0	6.9-8.1	11.4
	aqueous sediment extract	0.1	0.1	0.1	< 0.1	< 0.1	0.1
	bound residues	0.0	0.2	0.2	0.0	0.1	0.2
Sum of individual	**Dichlofluanid**	**99.4**	**49.2-57.6**	**4.3**	**99.2**	**34.9-51.8**	**9.4**
	DMSA	**0.5**	**35.5-46.2**	**90.9**	**0.7**	**42.4-59.3**	**84.4**