

Section A7.1.2.2.2 Water/sediment degradation study (02)**Annex Point IIIA XII2.1**

		1 REFERENCE
1.1	Reference	Scholz, K. (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), date: 1997-12-10. Hardy, IAJ and Patel, M (2005): Dichlofluanid: Kinetic Modelling Analysis of Data from Two Water Sediment Studies. Battelle UK Ltd., Report-No. CX/05/058, date: 2005-10-14.
1.2	Data protection	Yes
1.2.1	Data owner	Bayer Crop Science AG (Scholz 1997) / LANXESS Deutschland GmbH (Hardy 2005)
1.2.2	Companies with letter of access	LANXESS Deutschland GmbH
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: 890524ELB01
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

Official
use only

Section A7.1.2.2.2 Water/sediment degradation study (02)**Annex Point IIIA XII2.1**

3.2.1	Initial concentration - of reference substance	
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)
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

Section A7.1.2.2 Water/sediment degradation study (02)**Annex Point IIIA XII2.1**

		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 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	In the original report of Scholz (1997) the DT ₅₀ values were read from the degradation curves and also calculated with the MGA Software (ACSL Optimize, v1.2, 1996, USA) following first order reaction kinetics. The kinetic of the aerobic water and water/sediment systems with dichlofluanid was reevaluated in 2005, based on the results obtained by Scholz in 1997. The water/sediment residue data were entered into the computer programme ModelMaker 4.0 for optimisation of the degradation rate constant (K _p) and initial soil residue (M ₀). A simple first order, non-linear regression model was used to describe the behaviour of dichlofluanid in the aqueous, sediment phase and overall total system. Table A7_1_2_2_2-5 shows the recalculated DT ₅₀ 's for dichlofluanid.	X X
		4 RESULTS	
4.1	Degradation of test substance		
4.1.1	Graph	Provided in both reports (initial report and reevaluation report)	
4.1.2	Degradation	See tables A7_1_2_2_2-3, A7_1_2_2_2-4 and A7_1_2_2_2-5	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.	
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	
		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	

Section A7.1.2.2.2 Water/sediment degradation study (02)**Annex Point IIIA XII2.1**

		<p>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.</p>	
5.2	Results and discussion	<p>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.</p> <p>In the report for new kinetic modelling analysis of data, an excellent model fit to the data was obtained, with a high r^2 value (except for the sediment of the Angler-Weiher system), low $\text{Min } x^2$ errors, highly significant parameter estimations (except for the sediment of the Angler-Weiher system), and the residual plot showing random scatter of data.</p> <p>The recalculated DT_{50} values with ModelMaker, as geometric mean, for each experiment, were 2.0 hours in the systems consisting of water only and 1.8 hours, 1.0 hours and 2.8 hours in the aqueous phase, sediment and total system respectively, in the experiment with water/sediment.</p>	X
5.3	Conclusion	<p>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. 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.</p>	
5.3.1	Reliability	Reliability indicator: 1	
5.3.2	Deficiencies	No	

Section A7.1.2.2.2

Annex Point IIIA XII.2.1

Water/sediment degradation study (02)

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	09/12/2013
Materials and Methods	<p>3.3.12: The water/ sediment study itself was evaluated as part of the submission for dichlofluanid PT8 (wood preservative). Here only the kinetic treatment of the data was evaluated by the UK CA.</p> <p>3.3.12: The applicant has used SFO kinetics in the programme Modelmaker to calculate DT₅₀ values for the dissipation of dichlofluanid in the supernatant water (Experiment I) and to calculate dissipation DT₅₀ values in the supernatant water, sediment, and system (Experiment II). The approach was considered reasonable by the UK CA although it was noted that there was a paucity of data points for the water / sediment system (Experiment II) that bring in question the validity of the kinetic evaluation.</p>
Results and discussion	<p>4.1.2: No evaluation has been made of the data in Tables A7_1_2_2_2-1 to A7_1_2_2_2-4 although it was noted that timepoints at 4, 12, and 17 hours were omitted from Table A7_1_2_2_2-3 but were included in the kinetic analysis. The UK CA applied FOCUS degradation kinetics guidance to the analysis of the data.</p> <p>4.1.2: The DT₅₀ values reported in Table A7_1_2_2_2-5 were accepted except the values for sediment which were rejected based on insufficient data. It was also noted that the values for the natural water only were considered more robust than the sediment / water system due to the lack of data in the latter system</p>

Conclusion

5.2.

Experiment I

The modelling was checked independently by the evaluator using Modelmaker version 4 and within an EXCEL spreadsheet. The χ^2 values were less than 15% and the visual fitting was good in all cases after fitting with SFO kinetics. There was no improvement when FOMC kinetics were used. DT₅₀ values were the same or very similar to the applicant (evaluator values summarised below) and so the values presented by the applicant in Table A7_1_2_2_2-5 were accepted.

Test system	DT ₅₀ (days)	χ^2 error level (%)	Visual fitting
Hönniger Weiher (water only)	0.12	8.2	good
Angler Weiher (water only)	0.06	5.3	good

Experiment II

There were only three timepoints points for the water dissipation and whole system degradation curves. For the sediment dissipation curve there were only two timepoints and an assumption was made that there was instantaneous partition to the sediment at the start of the experiment. For a robust estimation of parameters, FOCUS kinetics recommends a minimum of six timepoints but does state that where degradation of the parent is very rapid that this may not be practical. The UK CA notes that although the degradation is indeed rapid, Experiment I showed that it was practical to obtain six timepoints before the parent had totally degraded.

The modelling was checked independently by the evaluator using Modelmaker version 4 and within an EXCEL spreadsheet.

Test system	compartment	DT ₅₀ (days)	χ^2 error level (%)	Visual fitting
Hönniger Weiher	water	0.11	15.3	poor
	sediment	0.09	10.3	good but only two timepoints
	system	0.13	16.5	poor
Angler Weiher	water	0.10	10.5	reasonable
	sediment	-	-	good but only two timepoints
	system	0.11	12.8	reasonable

Reliability

1

Acceptability

Acceptable (kinetic treatment of the data; caveats in the conclusion above)

Remarks

No other comments

Date**COMMENTS FROM ...***Give date of comments submitted*

Materials and Methods	<i>Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state</i>
Results and discussion	<i>Discuss if deviating from view of rapporteur member state</i>
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>
Reliability	<i>Discuss if deviating from view of rapporteur member state</i>
Acceptability	<i>Discuss if deviating from view of rapporteur member state</i>
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 CaCl ₂)	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-¹⁴C]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-¹⁴C]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

Table A7_1_2_2_2-5: DT₅₀ values for dichlofluanid calculated with ModelMaker in 2005

Compound	System	DT ₅₀ (days)		
		Aqueous phase	Sediment phase	Total system
Dichlofluanid	Natural water only taken from the Hönniger-Weiher test system	0.12 (2.9 hours)	-	-
	Natural water only taken from the Angler-Weiher test system	0.06 (1.4 hours)	-	-
	Geometric mean	0.09 (2.0 hours)	-	-
	Hönniger-Weiher	0.11 (2.6 hours)	0.09 (2.2 hours)	0.13 (3.1 hours)
	Angler-Weiher	0.05 (1.2 hours)	0.02 (0.5 hours)	0.11 (2.6 hours)
	Geometric mean	0.07 (1.8 hours)	0.04 (1.0 hours)	0.12 (2.8 hours)