

**Section A7.2.2.4. Anaerobic degradation in soil****Annex Point: IIIA XII 1.1**

		1 REFERENCE	Official use only  X
<b>1.1</b>	<b>Reference</b>	Scholz, K., 1988, Metabolism of [ring-UL-14C] dichlofluanid (Euparen®) in soil under anaerobic conditions, Bayer AG, Institute for Metabolism Research, Monheim, Germany, Report No. PF 2894, 1987-11-09.	
<b>1.2</b>	<b>Data protection</b>	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	
<b>2.1</b>	<b>Guideline study</b>	EPA Pesticide Assessment Guidelines § 162-2, October 1982	
<b>2.2</b>	<b>GLP</b>	No, GLP requirements of 40 DFR Part 160 do not apply to the study described.	
<b>2.3</b>	<b>Deviations</b>	No	
		3 MATERIALS AND METHODS	
<b>3.1</b>	<b>Test material</b>	a) [benzene ring-UL- <sup>14</sup> C] dichlofluanid 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, Elberfeld, Germany. b) as given in section 2 of dossier, sample provided by Bayer AG, Elberfeld, Germany	
3.1.3	Purity	a) [REDACTED] radiochemical purity b) [REDACTED] purity	
3.1.4	Further relevant properties	-	
3.1.5	Method of analysis	Soil was extracted with one portion of methanol/water and two portions of methanol. Extracts were combined, pooled radioassayed by LSC and analysed with HPLC and TLC. Analysing of bound residues: the soil samples, which had been extracted with solvents, were dried, ground in a mill and ashed in an automatic sample oxidizer.	
<b>3.2</b>	<b>Reference substance</b>	Dichlofluanid, Dimethylaminosulfanilide (DMSA), Methylaminosulfanilide (KUE 8630B), Amino sulfoanilide (KUE 9079A), 4-Hydroxydimethylaminosulfoanilide (KUE 86630A and KUE 8630C) and Phenylamido sulfonic acid (K-salt) (THS 3245)	
3.2.1	Method of analysis for reference	Dichlofluanid, Dimethylaminosulfanilide and Methylaminosulfanilide were extracted with methanol and measured by GC-MS.	

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	substance	
3.3	<b>Soil types</b>	One soil was used, see table A7_2_2_4-1
3.4	<b>Testing procedure</b>	
3.4.1	Test system	<p>Radioactive labelled dichlofluanid was dissolved in ethyl acetate and applied to soil screened to a particle size <math>\leq 2</math> mm via a subsample, resulting in a concentration of 9 mg/kg.</p> <p>Variant A: Incubation vessels for anaerobic soil metabolism studies were used; anaerobic conditions from the start of experiment. The 100 g soil samples were mixed with 80 ml of distilled water gasified with N<sub>2</sub> so that a layer of approx. 2 cm deep stood above the soil surface. The conical flasks were then flushed out with nitrogen, closed and stored in the dark at 22 °C (<math>\pm 2</math> °C). The flasks were sampled at day 30, day 61 and day 90;</p> <p>Variant B: For these samples, anaerobic degradation was preceded by a period of aerobic preincubation in vessels for aerobic soil metabolism studies (according to J.P.E. Anderson: Soil Biol. Biochem., 10, p. 215-221 (1978)). After 30 days aerobic preincubation, the soil samples switched to anaerobic conditions as described for variant A. The flasks were sampled at day 31 and 60.</p>
3.4.2	Test solution and Test conditions	Labelled and unlabelled dichlofluanid were dissolved and mixed; 100 $\mu$ l of the stock solution contained 0.87 mg a.i. (321.374 kBq).
		4 RESULTS
4.1	<b>Aerobic soil metabolism</b>	See table A7_2_2_4-2
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	<b>Materials and methods</b>	US EPA Guideline 162-2 was followed. The soil metabolism of [benzene ring-UL-14C] dichlofluanid under anaerobic conditions was investigated in a sandy loam soil. In a variation of the test, an aerobic preincubation of the samples was performed. The average concentration of dichlofluanid was 9 mg/kg soil
5.2	<b>Results and discussion</b>	
5.2.1	DT50 values	not determined
5.2.2	Degradation products (% of a.s.)	<p>In anaerobic soils dichlofluanid was rapidly degraded to dimethylaminosulfanilide (DMSA). After 30 days 87.4-95.5% of the parent compound was degraded to DMSA and the percentage of parent compound was less than 0.1%.</p> <p>Small amounts of methylaminosulfanilide (KUE 8630B) were also detected (<math>\leq 0.2\%</math>).</p>
5.2.3	Bound residues	<p>The bound residues after 30 days were at a level between 6.8% and 11.9%. At the end of the study 10.6-11.1% bound residues were found (after 90 d).</p> <p>In the variant with aerobic preincubation the proportion of bounded</p>

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		residues was distinctly lower than in the purely anaerobic systems (47.5-49.9% after 31 days).
5.2.4	CO <sub>2</sub> formation	The CO <sub>2</sub> formation in the biological active soils was very low ( $\leq 0.3\%$ ).
5.3	<b>Conclusion</b>	Under anaerobic conditions in soil dichlofluanid is rapidly degraded to dimethylaminosulfanilide (DMSA).
5.3.1	Reliability	2
5.3.2	Deficiencies	Batch numbers of test compound not given

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<b>Evaluation by Competent Authorities</b>	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	<b>EVALUATION BY RAPPORTEUR MEMBER STATE</b>
<b>Date</b>	31/08/05
<b>Materials and Methods</b>	1.1 The reference is correctly dated 1987. The applicant's version is acceptable.
<b>Results and discussion</b>	The applicant's version is acceptable with the following exception. 5.2.3 Applicant has wrongly summarised these results. The study and Table 7_2_2_4-2 (Degradation in soil under laboratory conditions) support the conclusion that the percentage of applied radioactivity present as bound residues in the totally anaerobic systems (6.8 - 20.5 %) were distinctly lower than that reported for the aerobic pre-incubation systems (47.5 - 60 %). The applicant has stated the opposite which is incorrect.
<b>Conclusion</b>	The applicant's version is acceptable.
<b>Reliability</b>	2
<b>Acceptability</b>	Acceptable
<b>Remarks</b>	All endpoints and data presented in the summary and tables have been checked against the original study and are correct.
	<b>COMMENTS FROM ...</b>
<b>Date</b>	<i>Give date of comments submitted</i>
<b>Materials and Methods</b>	<i>Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state</i>
<b>Results and discussion</b>	<i>Discuss if deviating from view of rapporteur member state</i>
<b>Conclusion</b>	<i>Discuss if deviating from view of rapporteur member state</i>
<b>Reliability</b>	<i>Discuss if deviating from view of rapporteur member state</i>
<b>Acceptability</b>	<i>Discuss if deviating from view of rapporteur member state</i>
<b>Remarks</b>	

**Table A7\_2\_2-4-1: Classification and physico-chemical properties of the soil used**

	Soil
Location	Stanley Research Center, Kansas City, USA
Soil texture	sandy loam
Sand [%]	67
Silt [%]	27
Clay [%]	6
Organic carbon [%]	4.6
pH (0.01 M CaCl <sub>2</sub> )	5.2
Biomass at start of study [mg microbial C/kg dry weight soil]	268

**Table A7\_2\_2\_4-2: Degradation in soil under standard laboratory conditions**

	Variant A: anaerobic degradation			Variant B anaerobic degradation with aerobic preincubation	
	30	61	90	30/31(aerobic / anaerobic)	30/60(aerobic / anaerobic)
Dose [mg/kg soil]	9			9	
Incubation [days]	30	61	90	30/31(aerobic / anaerobic)	30/60(aerobic / anaerobic)
Dichlofluanid [%]	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
DMSA [%]	87.4-95.5	80.4-89.1	88.1-88.3	35.2-35.6	28.3-28.8
KUE 8630B [%]	< 0.1-0.2	< 0.1	< 0.1	8.1	7.1-7.5
Not identified [%]	< 0.1-0.1	0.1	< 0.1	0.6-0.8	2.1
<sup>14</sup> CO <sub>2</sub> (headspace + water) [%]	< 0.1	0.1-0.3	< 0.1-0.1	3.4-3.6	5.1-7.1
<sup>14</sup> CH <sub>4</sub> [%]	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Bound residues	6.8-11.9	8.1-20.5	10.6-11.1	47.5-49.9	58.7-60.0
Total recovered radioactivity [%]	99.6-102.3	97.4-101.3	99.0-99.4	95.4-97.4	103.1-103.8