Section A7.2.1 Aerobic degradation in soil

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			Official use only
		1 REFERENCE	
1.1	**Reference**	Scholz, K., 1988, Metabolism of [benzene-ring-UL-14C] dichlofluanid (Euparen®) in soil under aerobic conditions, Bayer AG, Institute for Metabolism Research, Monheim, Germany, Report No. PF 2985, 1988-01-25.	
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**	EPA Pesticide Assessment Guidelines § 162-1, October 1982	
2.2	**GLP**	No, GLP requirements of 40 DFR Part 160 do not apply to the study described.	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	a) [benzene ring-UL-^{14}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 methanol/water and dichlormethane. Extracts were pooled radioassayed by LSC and analysed with HPLC and TLC. Analysing of bound residues: the soil was treated with 0,5 M NaOH and extracted for 24 hours. After centrifugation the radioactivity in the sediment was determined by ashing (humin). To precipitate the humic acid fraction the supernatant was acidified with HCl to a pH of 2. The radioactivity of the supernatant (fulvic acid) and the sediment taken up in 0.5 M NaOH (humic acid) was determined. The quantification of the humic acid, fulvic acid and humin fraction was done with LSC. Verification of microbial activity was accomplished by monitoring the evolved $^{14}CO_2$ from 100 g soil. Seperate batches were available to detect	X

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		the microbial biomass at the start of the test and at certain sampling points. CO_2 trapping solutions were radioassayed by LSC.	
3.2	**Reference substance**	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 substance	Dichlofluanid, Dimethylaminosulfanilide and Methylaminosulfanilide were extracted with methanol and measured by GC-MS	
3.3	**Soil types**	Three soil types were used, see table A7_2_1-1	**X**
3.4	**Testing procedure**		
3.4.1	Test system	Incubation vessel for aerobic soil metabolism studies (according to J.P.E. Anderson: Soil Biol. Biochem., 10, p. 215-221 (1978)). Radioactive labelled dichlofluanid was dissolved in ethyl acetate and applied to 100 g soil screened to a particle size ≤ 2 mm via a subsample, resulting in a concentration of 10 mg/kg. Then incubated in glass flasks with CO_2-trap under aerobic conditions in the dark at 23 ± 2 °C. The flasks were sampled at day 1, 3, 8, 14, 30, 59, 97 181 (Variant 1a); at day 0, 1, 3, 8 (Variant 1b and 1c); at day 8, 30, 90, 181 (Variant 2); at day 0, 30, 61, 90 183 (Variant 3); at day 0, 30, 58, 97, 132, 181, 280 and 414 (Variant 4). In test variant 2 (with steril soil) the parent compound solution was dripped onto the sterile soil under sterile conditions.	
3.4.2	Test solution and Test conditions	A separate stock solution was prepared for each soil type. The radioactive labelled dichlofluanid was dissolved in ethyl acetate and mixed with unlabelled parent compound.	
		4 RESULTS	
4.1	**Aerobic soil metabolism**	See table A7_2_1-2	**X**
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	US EPA Guideline 162-1 was followed. The soil metabolism of [benzene ring-UL-14C] dichlofluanid under aerobic conditions was investigated in two sandy loam soils (soil 1 and 3) and a sand soil (soil 2). In a variation of the test, work was performed with sterile soil 2. The average concentration of dichlofluanid was 10 mg/kg soil.	
5.2	**Results and discussion**		
5.2.1	DT50 values	In biological active soils the half-life of dichlofluanid was less than one day (DT50 < 1 day). In the sterile soil after 90 days still 53.2% of dichlofluanid were present (DT50 > 90 days).	X
5.2.2	Degradation	Dichlofluanid was rapidly degraded by biological active soils to	

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	products (% of a.s.)	dimethylaminosulfanilide (DMSA). After 1 day 79.5-84.0% of the parent compound was degraded to DMSA. After 90 days the percentage of parent compound was less than 0.1% in the living soils. Beside dimethylaminosulfanilid (DMSA) a further metabolite could be identified as methylaminosulfanilide (KUE 8630B). This metabolite reached his highest concentration (8.2%) in soil 1 after 97 days.
5.2.3	Bound residues	The bound residues in the living soils after 30 days were at a level between 24.2% and 42.5%. At the end of the study 56% bound residues were found in soil 1 (after 181 d), 69.4 in soil 3 (after 183 d) and 75.7% in soil 4 (after 414 d), respectively. In the sterile variant (soil 2) only max. 4.8% of the applied radioactivity was found in the bound residues fraction (after 181 d). Dimethylaminosulfanilide and small quantities of methylamino-sulfanilide could be released from this residue after hydrochloric acid/acetone extraction.
5.2.4	CO_2 formation	The CO_2 formation in the biological active soils was 9.2% (soil 3) to 22.6% (soil 4) at the end of the experiments (183 and 414 days, respectively). Under sterile soil conditions a CO_2 formation of only 0.2% of applied radioactivity was detected after 181 days.
5.3	**Conclusion**	Dichlofluanid is rapidly degraded in biological active soils to dimethylaminosulfanilide (DMSA). Under such conditions the half-life of dichlofluanid is less than one day (DT50 < 1 day). In sterile soil the degradition of dichlofluanid is much slower (DT50 > 90 days).
5.3.1	Reliability	2
5.3.2	Deficiencies	Batch numbers of test compound not given

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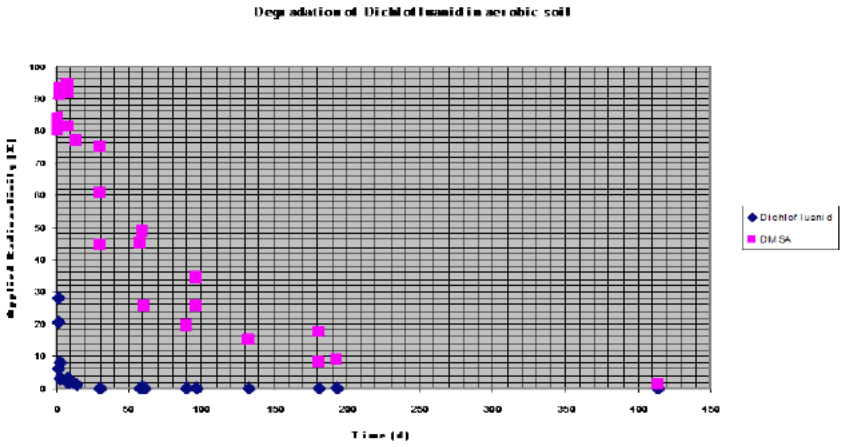
Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	13/07/2006
Materials and Methods	The applicant's version is acceptable with the following amendments and comments. **3.1.5** Typo: 0,5 M NaOH should read 0.5 M NaOH. **3.3** Table A7_2_1-1 No details are given in the row for Cation Exchange Capacity – these are not available from the report and row therefore should be deleted.
Results and discussion	The applicant's version acceptable with the following amendment. **4.1** Table A7_2_1-2 For variant 4, the total recovered radioactivity after 414 days exposure should read 101.6 %. **5.2.1** Using the data available, the half-life of DMSA can be estimated (graphically) at approximately 40 days assuming that the maximum concentration was reached by day 3 (see figure below). This is at a maximum of 25 °C, which would equate to approximately 113 d at 12 °C and give a degradation rate constant of 0.00613 (TDG eq. 25 and 29).
Conclusion	The applicant's version is acceptable.
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 unless highlighted above.



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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_2_1-1: Classification and physico-chemical properties of soils used

	Soil 1	Soil 2	Soil 3
Name	Speyer II standard soil (= BBA soil 2.2)	Speyer I standard soil (= BBA soil 2.1)	Kansas
Location	Hanhofen, Germany	Jockgrim, Germany	Stanley Research Center, Kansas City, USA
Soil texture	sandy loam	sand	sandy loam
Sand [%]	80	87	67
Silt [%]	12	9	27
Clay [%]	8	4	6
Organic carbon [%]	2.6	0.8	1.3
pH (0.01 M $CaCl_2$)	7.1	5.4	5 2
Cation exchange capacity (MEQ/100 g at pH 8.2)			
Biomass at start of study [mg microbial C/kg dry weight soil]	340	90	243

Table A7_2_1-2: Degradation in soil under standard laboratory conditions

	Variant 1a: Soil 1 (living)	Variant 2: Soil 1 (sterile)	Variant 3: Soil 2 (living)	Variant 4 Soil 3 (living)
Dose [mg/kg soil]	10	10	10	10
Incubation [days]	181	181	183	414
Dichlofluanid [%]	< 0.1	49.7	< 0.1	< 0.1
DMSA [%]	17.8	46.1	8.5	1.4
KUE 8630B [%]	7.3	-	2.4	1.2
Not identified [%]	1.8	-	3.5	0.7
$^{14}CO_2$ [%]	10.9	0.2	9.2	22.6
Bound residues	56.0	4.8	69.4	75.7
a. Fulvic acid	22.7			
b. Humic acid	17.6			
c. Humin	11.9			
Total recovered radioactivity [%]	93.8	100.8	93.0	