Section A7.2.3.2 Soil leaching study

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			Official use only
		1 REFERENCE	
1.1	**Reference**	Scholz, K., 1987, Leaching characteristics of Dichlofluanid (Euparen®) with various modes of application. Bayer AG, Institute for Metabolism Research, Monheim, Germany, Report No. PF 2799, 1987-05-18.	
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, Federal German Biological Agency for Agriculture and Forestry (BBA), Bulletin No. 37, 3rd edition (1984).	
2.2	**GLP**	GLP requirements of 40 CFR Part 160 do not apply to the study described in this document	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	a) [U^{14}C-phenyl]-dichlofluanid b) 50 WP [U^{14}C-phenyl]-dichlofluanid c) non-active standard substance (dichlofluanid)	
3.1.1	Lot/Batch number	Not given	
3.1.2	Specification	a) specific radioactivity was 1247 kBq/mg; sample provided from Bayer AG, Isotope Laboratory, Elberfeld, Germany, b) specific radioactivity was 623 mBq/mg; sample provided from Bayer AG, Isotope Laboratory, Elberfeld, Germany, c) as given in section 2 of dossier, sample provided by Bayer AG, Elberfeld, Germany	
3.1.3	Purity	a) [redacted] radiochemical purity b) [redacted] radiochemical purity c) [redacted] purity	
3.1.4	Further relevant properties	50 WP (Wettable Powder) is an agricultural formulation of dichlofluanid.	
3.1.5	Method of analysis	Fraction I (the first 200 ml of leachate) was not subjected to further analysis because the radioactivity level was too low. Fraction II (the second 200 ml) from variants 2B and 3A was extracted twice with chloroform. The chloroform phase was concentrated on a rotary evaporator, taken up in 1ml chloroform, and analysed using Thin Layer Chromatography. Determination of ^{14}C Radioactivity: The volatile compounds were	X

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		trapped in a oil-coated wool plug, extracted and measured by LSC. The $^{14}CO_2$ was passed into a cocktail and measured by LSC. The liquid samples were analysed by LSC too. The soil samples were incinerated in an automatic combustion machine.	
3.2	**Reference substance**	Yes, Dimethylaminosulphanilide (DMSA), the main metabolite was analysed in parallel as a reference compound.	**X**
3.2.1	Method of analysis for reference substance	TLC; the TLC findings were confirmed by mass-spectrometric analysis.	
3.3	**Soil types**	One soil was used (BBA Standard Soil 2.1), see table A7_2_3_2-1	**X**
3.4	**Testing procedure**		
3.4.1	Test system	The soil was sieved to a particle size ≤ 1 mm; three experimental variants were performed (see table A7_2_3_2-2). *Experimental variants:* In variant 1 the WP formulation was suspended in 1ml water and applied drop wise to the surface of the leach column. In variant 2 the WP formulation was worked into the soil via a soil sub sample. In variant 3 the unformulated active ingredient was worked into the soil via a soil sub sample. *Leaching:* Two columns were prepared with BBA standard soil 2.1 according to BBA bulletin 37 (height of soil column after compression 26 cm). The columns were watered with about 400 ml of water over a period of 48 h. The leachate was collected in 2 fractions of 200 ml. When the watering was finished the soil columns were deep-frozen. The columns from variants 1B, 2B and 3A were sliced into 3 pieces of equal length.	
3.4.2	Test solution and Test conditions	The WP formulation was suspended in water (variants 1 and 2) In variant 3 the radioactive labelled dichlofluanid was dissolved in ethyl acetate and mixed with unlabelled parent compound. The application rate (0.5 mg/column) was based on the maximum rate used in agricultural practice (2.5 kg/ha). This application rate is equivalent to 0.5 mg active ingredient per column.	
		4 RESULTS	
4.1		See table A7_2_3_2-3	**X**
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Dichlofluanid WP 50 formulation and unformulated active ingredient were dropped on the surface of soil columns or sub samples of treated soil were transferred to the top of soil columns containing untreated sand soil according to BBA Bulletin 37. The application rate was 0.5 mg dichlofluanid per column. The columns were then leached for 48 h with 400 ml water and the leachate was collected in 2 fractions of 200 ml.	

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5.2	**Results and discussion**	After drop wise application of the suspended formulation on the surface of the soil in the leach-column, the leachate contained fewer radioactivities in the 2-day test than after incorporation of the formulation into the upper part of the soil column. The percentage of radioactivity in the leachate was less for the trial with incorporated formulation than for the trial with non-formulated incorporated compound. The leachate contained no unchanged parent compound (< 0.1 %); the major proportion was dimethylaminosulfanilide (DMSA). DMSA occurred in the leachate in amounts ranging from 1% (formulation, not incorporated) to 30% (active ingredient, incorporated) of the originally applied radioactivity, depending on the method of application used.	X
5.3	**Conclusion**	Dichlofluanid can be classified as an immobile compound.	
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	11/10/2004
Materials and Methods	The applicant's version is acceptable with the following comments and amendments. **3.1.5** Agree with first paragraph. From second paragraph onwards this is incorrect and likely to reflect a short cut edit error (cut and paste of previous study). The actual methodology for the determination of ^{14}C radioactivity according to the original report has been summarised below (*italics*): *Radioactive substances were separated using silica gel plates (Merc silica gel 60F) and 8:2 hexane:glacial acetic acid as the solvent. DMSA was used as a reference compound and the corresponding silica gel plate removed, extracted with ethyl acetate, centrifuged and evaporated to dryness with N_2 (22°C). The sample was then mixed with N-methyl-N-trimethyl-silytrifluroacetamide-acetonitrile (1:1) and incubated for 1 hour at 70°C. The mass spectrometry analysis was then performed directly on the reaction mixture without further working up.* *The ^{14}C-radioactivity was determined before and after each working step. The liquid samples were analysed using liquid scintillation counting (LSC). The solid samples were air-dried, mixed (star mix 5 min) and 100g sample removed and ground for 10 min (Retsch mill). Sub samples were then ashed in an automatic sample oxidiser to determine the radioactive content.* *The TLC plates were evaluated the radioactive zones being compared to reference compounds, and the substance spots were visualised by:* *1) Extinction of the UV-induced fluorescence of TLC plates (254 nm) in the case of UV absorbing substances.* *2) Linear analyser with evaluator unit* *3) Autoradiography on Agfa curix clear base X-ray film.* **3.2** Applicant has stated YES, but has not specified the reference substance. However, the UK CA considers that the Applicant is referring to the use of a reference standard for the analytical method as no reference substance was used in the actual leaching study. **3.3** The soil characteristics given in Table A7_2_3_2-1 give details of the texture as sand, which is additional to the information provided within the original study. However, the data given have been accepted by UK CA as the soil used is a standard German Speyer soil for which these characteristics have been confirmed.

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Results and discussion

The applicant's version is accepted with the following amendment.

4.1: The following corrections to the data presented in Table A7_2_3_2-3 should be noted.

1.

		1A
Leachate	Fraction I	< 0.001
	Fraction II, total	0.4

2.

		3A
Leachate	*Unidentified radioactivity*	< 0.1

5.2 The Applicant's statement that 'DMSA occurred in the leachate in amounts ranging from 1% (formulation, not incorporated)' is incorrect as Table A7_2_3_2-3 shows, this should therefore read 'DMSA occurred in the leachate in amounts ranging from 10% (formulation, incorporated)'.

Conclusion

The applicants version is acceptable with the following comments.

This study is acceptable to demonstrate the conclusions reached. In addition to the conclusion regarding the mobility of dichlofluanid, this study also demonstrates the rapid degradation to DMSA, which depending on the mode of application can be classified as immobile to slightly mobile.

Reliability 2

Acceptability Acceptable

Remarks All endpoints and data presented in the summary and tables have been checked against the original summary and are correct unless stated above.

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_3_2-1: Classification and physico-chemical properties of the soil used

	BBA Standard Soil 2.1
Location	Speyer, Germany
Soil texture	sand
Suspendable fraction [%]	10.7
Organic carbon [%]	0.69
pH (0.01 M $CaCl_2$)	7.0
Max. water capacity [%]	18.2
Biomass at start of study [mg microbial C/kg dry weight soil]	106

Table A7_2_3_2-2: Experimental variants of the leaching experiment and radioactivity in the leachates

Experi-mental variant	**Experimental conditions**				**Result**
	Method of application	**mg a.i/ soil column**	**mg product/ soil column**	**Quantity of radioactivity applied [kBq]**	**Radioactivity in leachate [% of applied radioactivity]**
1 A	50 WP in 1 ml water applied drop wise to soil	0.49	0.98	610.6	0.4
1 B		0.50	1.00	623.3	1.3
2 A	50 WP worked into the soil via a sub sample	0.49	0.97	605.2	7.6
2 B		0.49	0.98	610.2	11.5
3 A	a.i. worked into the soil via a sub sample	0.49	-	40.9	32.4
3 B		0.49	-	40.9	28.1

Table A7_2_3_2-3: Distribution of dichlofluanid residues following soil column leaching. Figures are in % of radioactivity applied to column (= 100 %)

		Experimental variant					
Radioactivity		**1 A**	**1 B**	**2 A**	**2 B**	**3 A**	**3 B**
I. Soil	upper third	--	78.9	--	52.2	16.3	--
	middle third	--	10.6	--	14.5	23.3	--
	lower third	--	5.1	--	13.7	28.2	--
II. Leachate	total	0.4	1.3	7.6	11.5	32.4	28.1
	Fraction I	--	0.01	< 0.1	0.2	0.1	< 0.1
	Fraction II, total	--	1.3	7.6	11.3	32.3	28.1
	Dichlofluanid in Fraction II	--	--	--	*< 0.1*	*< 0.1*	--
	Dimethylaminosulfanilide in Fraction II	--	--	--	*10.0*	*32.0*	--
	Unidentified Radioactivity	--	--	--	*< 0.1*	*0.1*	--
	Aqueous phase after extraction	--	--	--	*1.3*	*0.3*	--
Sum I. + II. [%]		--	95.9	--	91.9	100.2	--
Sum I. + II. [kBq]		--	597.7	--	560.8	41.0	--
Total applied radioactivity [kBq]		610.6	623.3	605.3	610.2	40.9	40.9

--: This experimental variant was not investigated