Section A6.2 Toxicokinetics

Annex Point IIA6.2 6.2 Metabolism of [ring-U-^{14}C]dichlofluanid in rats

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted], 1986, Structural clarification of metabolites of [ring-U-^{14}C]dichlofluanid in rat faeces, [redacted], PF Report No. [redacted], 1986-11-17 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Bayer CropScience 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**	No Methods used in this study are comparable to OECD-Guideline 417.
2.2	**GLP**	No GLP was not compulsory at the time the study was performed.
2.3	**Deviations**	Yes - No quantitation of metabolites was provided.
		3 MATERIALS AND METHODS
3.1	**Test material**	
3.1.1	**Non-labelled parent compound**	Not used.
3.1.2	Lot/Batch number	—
3.1.3	Specification	—
3.1.3.1	Description	—
3.1.3.2	Purity	—
3.1.3.3	Stability	—
3.1.4	**Labelled parent compound**	[ring-U-^{14}C]dichlofluanid
3.1.5	Lot/Batch number	[redacted]
3.1.6	Specification	—
3.1.6.1	Description	—
3.1.6.2	Purity	Radiochemical and chemical purity [redacted] Radiochemical purity tested by TLC and HPLC and the chemical purity was confirmed by GC.

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3.1.6.3	Stability	The compound was stable for at least 4 hours (in the application solution) as tested by means of thin-layer-chromatography (TLC, silicagel; aceton 20/n-hexane 40). The concentration of each administration was checked radiometrically; these measurements served as a reference of the radioactivity in the different biological samples.
3.1.6.4	Radiolabelling	^{14}C-labelled in the benzene ring. [ring-U-^{14}C]dichlofluanid
3.1.6.5	Reference compounds	The following substances were used as reference compounds (for structural formula see appendix 1): KUE 8630A, KUE 8630B, KUE 8630C, KUE 9079A and N'-dimethyl-N-phenylsulfuric acid diamide (DMSA).
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Sprague-Dawley
3.2.3	Source	[redacted]
3.2.4	Sex	Male
3.2.5	Age/weight at study initiation	Age: young adults Weight: 210.2 and 209.9 g
3.2.6	Number of animals per group	2 animals
3.2.7	Control animals	No
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	Single application.
3.3.2	Post-exposure period	2 days.
3.3.3	Specific activity of test substance	[Ring-U-^{14}C]-dichlofluanid: 33.7 µCi (1.247 MBq/mg), which corresponds to 11.23 mCi/mmol (415.47 MBq/mmol)
3.3.4	Type	Gavage
3.3.5	Concentration of test substance	10 mg/kg bw.
3.3.6	Vehicle	Suspension composed of 0.9% sodium chloride, 10% Cremophor EL, and 0.1% active substance
3.3.7	Concentration in vehicle	1 mg/ml
3.3.8	Volume applied	2.0 ml (= 2.0 mg active substance)
3.4	**Examinations**	
3.4.1	Biokinetic parameters	Metabolism.
3.3.9	Samples	Faeces
3.3.10	Sampling time	0 –24 h and 24 – 48 h after administration.

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		4 RESULTS AND DISCUSSION	
4.1	**Toxic effects, clinical signs**	Not described.	
4.2	**Recovery of labelled compound**	After the oral application of 20 mg [ring-U-^{14}C]-dichlofluanid/kg bw to male and female rats 3.1 to 8-6 % of the retrieved radioactivity was excreted with the faeces. Reference: [redacted], 1985, [Phenyl-UL-^{14}C]dichlofluanid biokinetics part of general metabolism study on rats, [redacted], PF-Report No. [redacted], 1985-07-01 (unpublished)	X
4.3	**Metabolites**	Five metabolites could be identified (see also appendix 2): KUE 8630A, KUE 8630B, KUE 8630C, KUE 9079A and N'-dimethyl-N-phenylsulfuric acid diamide (DMSA).	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The metabolism of dichlofluanid was examined in rats serving as a model for mammals. For this purpose the faecally excreted metabolites were examined. The methods in this study used were comparable with the OECD-Guideline 417. For the current investigation [ring-U-^{14}C]dichlofluanid was used, which was labelled with ^{14}C in the benzene ring. [Ring-U-^{14}C]dichlofluanid was administered orally in 10% aqueous Cremophor EL solution with 0.9% common salt at single dose levels of 10 mg/kg bw to two adult male Sprague-Dawley rats. Faeces were collected during the period of 0 –24 hours and 24 – 48 hours post application, freeze dried, and homogenised. Faecal samples were extracted with methanol and subsequently analysed for metabolites by AMD-TLC (automated multiple development thin layer chromatography) or further extracted in a toluene/water system for gel permeation chromatography. An aliquot of the methanol extract was dried and digested with β-glucuronidase/arylsulphatase to identify conjugates. This solution was further investigated by AMD-TLC and gel permeation chromatography. To determine the metabolite distribution of dichlofluanid in the rat, the methanol extracts were analysed by AMD-TLC in two different solvent systems and identified through co-chromatography and over-spotting the samples with reference compounds. The toluene extracts and gel permeation fractions were further analysed by gas chromatography and mass spectrometry (GC-MS) or the fractions of the gel permeation chromatography were subsequently analysed by AMD-TLC or GC-MS.	

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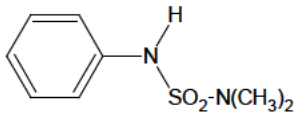
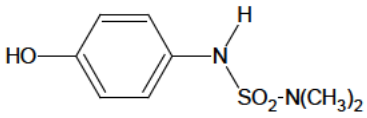
5.2	**Results and discussion**	Automated multiple-development TLC (AMD-TLC) of the crude faeces extract suggested that the metabolites consists mainly of compounds having the structures KUE 8630 A –C, KUE 9079 A, and DMSA (for formula see appendix 1). In these investigations the two hydroxylated compounds KUE 8630 A and C were detected in their unconjugated forms. Since they were definitely excreted from the animal organism as conjugates, cleavage must have occurred either during extraction or during AMD-TLC. Metabolites of the above structures were identified by GC-MS investigations on suitably pre-purified extracts. The following metabolic pathway was suggested (see appendix 2): These results show that dichlofluanid is initially degraded to N'-dimethyl-N-phenylsulfuric acid diamide (DMSA). Further biotransformation then occurs at two positions in the molecule: 1. successive demethylation of the dimethylamino group and 2. hydroxylation in position 4 of the benzene ring.
5.3	**Conclusion**	
5.3.1	Reliability	2
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	09/08/06
Materials and Methods	The UK CA notes that only one dose level was used in this study, and not two as specified in the current OECD TG. The dose level was 10 mg/kg/day and not 20 mg/kg/day as detailed in Section 4.2 above. However, the study has still provided useful information on the toxicokinetics of dichlofluanid.
Results and discussion	As described above
Conclusion	As described above
Reliability	2
Acceptability	Acceptable
Remarks	The UK CA agrees with applicant's summary.
	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	

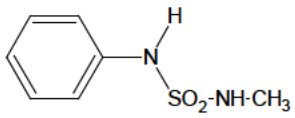
Appendix 1: Reference compounds used in the metabolic study of [ring-U-^{14}C]-dichlofluanid.



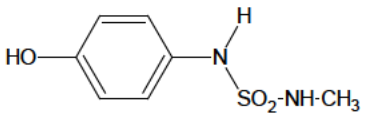
DMSA



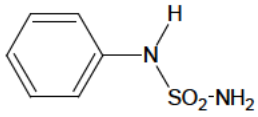
KUE8630A



KUE8630B



KUE8630C



KUE9079A

Appendix 2: Results of the metabolic study in rats: metabolic pathway of [ring-U-^{14}C]-dichlofluanid.