Section A6.2 Toxicokinetics

Annex Point IIA6.2 6.2 Metabolism of [^{14}C]dichlofluanid in the rat

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] 1978, Biotransformation of [^{14}C]dichlofluanid in the rat, [redacted]. Pharma-Report No. [redacted], PF-Report No. [redacted], 1978-08-14 (unpublished)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 No guidelines were available at the time the study was performed.
2.2	**GLP**	No GLP was not compulsory at the time the study was performed.
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	
3.1.1	**Non-labelled parent compound**	Dichlofluanid technical
3.1.2	Lot/Batch number	—
3.1.3	Specification	—
3.1.3.1	Description	—
3.1.3.2	Purity	[redacted] Determination by GC and TLC
3.1.3.3	Stability	—
3.1.4	**Labelled parent compound**	[Fluorodichloromethyl-^{14}C]dichlofluanid
3.1.5	Lot/Batch number	—
3.1.6	Specification	—
3.1.6.1	Description	—

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3.1.6.2	Purity	Approx. [redacted] (radiochemical); Determination by GC and TLC
3.1.6.3	Stability	—
3.1.6.4	Radiolabelling	^{14}C labelling at C1 (carbon of the fluorodichloromethylmercapto group) [Fluorodichloromethyl-^{14}C]dichlofluanid
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Sprague-Dawley, (SPF)
3.2.3	Source	[redacted]
3.2.4	Sex	Males
3.2.5	Age/weight at study initiation	Age: adult Weight: approx. 200 g
3.2.6	Number of animals	Not reported.
3.2.7	Control animals	No
3.3	**Administration/ Exposure**	Oral or intravenous
3.3.1	Duration of treatment	Single application
3.3.2	Post-exposure period	8 hours
3.3.3	Specific activity of test substance	58.4 µCi/mg (= 2.16 MBq/mg)
3.3.4	**Oral application**	
3.3.5	Type	Not reported.
3.3.6	Concentration of test substance	5 or 10 mg/kg bw
3.3.7	Vehicle	5 % aqueous Cremophor EL solution with 0.9 % common salt
3.3.8	Concentration in vehicle	—
3.3.9	Volume applied	—
3.3.10	**Intravenous application**	
3.3.10.1	Vehicle	5 % aqueous Cremophor EL solution with 0.9 % common salt
3.3.10.2	Concentration of test substance	5 or 10 mg/kg bw
3.3.10.3	Concentration in vehicle	—
3.3.10.4	Total volume applied	—
3.4	**Examinations**	

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3.4.1	Biokinetic parameters	Metabolism
3.4.2	Samples	Urine
3.4.3	Sampling time	Urine: 0 – 8 hours post application of the radiolabelled substance.

4 RESULTS AND DISCUSSION

4.1	**Toxic effects, clinical signs**	No toxic effects described.
4.2	**Recovery of labelled compound**	After oral or intravenous application of 10 mg [Fluorodichloromethyl-^{14}C]-dichlofluanid/kg bw to male rats, the bulk of the total amount of renally eliminated radioactivity, i.e. 40-50% of the applied dose, was found to have been excreted in the urine after 8 hours. Further examinations were not performed.
4.3	**Metabolism**	Dichlofluanid was metabolised extensively by rats. Following both the intravenous and oral application of 10 mg [Fluorodichloromethyl-^{14}C]-dichlofluanid/kg bw, the main radioactive metabolite was TLC-identical with the reference compound TTC (thiazolidine-2-thione-4-carboxylic acid) in two different solvent systems. HPLC comparisons of urine obtained after intravenous and oral application of 5 mg [Fluorodichloromethyl-^{14}C]-dichlofluanid/kg bw showed that the detected main radioactive metabolite was identical with TTC in three different HPLC systems and after both modes of application. The parent compound Dichlofluanid was not detected in any instance. Because of the results obtained in this study and the results of biotransformation studies performed with captan and folpet (De Baun et al., Xenobiotica 4 (1974) 101; Couch, R. C. & Siegel, M. R., Pestic. Biochem. Physiol. 7 (1977), 531; Couch et al. Pestic. Biochem. Physiol. 7 (1977), 547; Lukens, R. J. & Sisler, H. D. Phytopathology 48 (1958), 235) the following metabolic pathway was suggested (see table A6_2-1.A): Step 1: A thiol group-containing compound like cystein, glutathione, coenzyme A, separates the trihalogenmethylmercapto group from the active ingredient molecule with formation of disulphide. The liberated dimethylaminosulfamide was not further investigated. Step 2: The mixed disulphide reacts with another molecule of a thiol group-containing compound and liberates the fluorodichloromethyl sulfenic acid. Step 3: The unstable sulfenic acid stabilises, with cleavage of hydrogen halide, to form dihalogen thiocarbonyl. This may continue to react as follows: Step 4: With water to form carbon oxysulfide or carbon dioxide; Step 5: With cystein to form thiazolidine-2-thione-4-carboxylic acid (TTC); Step 6: With glutathione to form the corresponding TTC derivative which then in Step7: hydrolyses to TCC and is eliminated. This step may under certain circumstances be catalysed by the enzyme γ-glutamyltranspeptidase and cystein glycinase which are involved in the conjugation of foreign substances with glutathione and the resultant mercapturic acid conjugates (James, S. P. & Pheasant, A. E., Xenobiotica 8 (1978) 207).

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This metabolic pathway does not preclude other thiol group-containing endogenous substances than cystein or glutathione as reaction partners such as cys-SH groups in structurally bound proteins that react with the intermediately formed dihalogen thiocarbonyl to form structurally bound TTC derivatives, undergo degradation at a rate depending upon their biological half-life, and may lead to late elimination of TTC.

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 the current investigation [Fluorodichloromethyl-^{14}C]-dichlofluanid was used. Dichlofluanid was labelled with ^{14}C at the carbon of the fluorodichloromethylmercapto group. It was administered orally or intravenously in a 5 % aqueous Cremophor EL solution with 0.9 % common salt at single dose levels of 5 or 10 mg/kg bw to adult Sprague-Dawley rats. Radioactivity was measured in 0 to 8-hours urine. The metabolites of dichlofluanid in the rat were quantified and identified on the one hand by thin –layer chromatography (TLC) and on the other hand by high-performance liquid chromatography (HPLC) and by co-chromatography and over-spotting the urine samples on reference compound thiazolidine-2-thione-4-carboxylic acid (TTC).
Analysis of urine obtained after intravenous or oral application of 5mg [Fluorodichloromethyl-^{14}C]-dichlofluanid/kg bw was performed in three different HPLC systems: reversed-phase HPLC and two different ion-pair HPLC.
Analysis of urine obtained after intravenous or oral application of 10 mg [Fluorodichloromethyl-^{14}C]-dichlofluanid/kg bw was performed by TLC in two different solvent systems.

5.2 Results and discussion

Dichlofluanid was metabolised extensively by rats. The main metabolite identified was thiazolidine-2-thione-4-carboxylic acid (TTC). The parent compound dichlofluanid was not detected in any instance. These data suggest cleavage of the fluorodichloromethylmercaptogroup from the parent molecule dichlofluanid.

5.3 Conclusion

5.3.1 Reliability: 3

5.3.2 Deficiencies: Yes,

Study was poorly reported and no quantitation of metabolites was provided.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	17/09/04
Materials and Methods	As described above [IUCLID 5.0 4/4]
Results and discussion	As described above
Conclusion	As described above
Reliability	3
Acceptability	Acceptable
Remarks	The UK CA agrees with the 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	

Table A6_2-1 Proposed metabolic pathway of [fluorodichloromethyl-^{14}C]-dichlofluanid in rats