Section A6.2 Toxicokinetics

Annex Point IIA6.2 6.2 Biokinetic behaviour in the rat after single application

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] 1977, [^{14}C]Dichlofluanid (active ingredient of ®Euparen) Biokinetic study on rats, [redacted], Report No. [redacted], 1977-10-26 (unpublished)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 The 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 Compared to OECD-Guideline 417 the following deviations could be ascertained: - Chemical purity of the radiolabelled test compound was not reported. - Results of the bile duct cannulation experiment and the results of the activity measurement in exhaled air were poorly reported.

3 MATERIALS AND METHODS

3.1	**Test material**	Data documented by [redacted]; Pharma-Report No. [redacted] Reference: [redacted], 1978, Biotransformation of [^{14}C]dichlofluanid in the rat, [redacted], Pharma-Report No. [redacted], PF-Report No. [redacted], 1978-08-14 (unpublished)
3.1.1	**Non-labelled parent compound**	Dichlofluanid tech.
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	—

Section A6.2 Toxicokinetics

Annex Point IIA6.2 6.2 Biokinetic behaviour in the rat after single application

3.1.6	Specification	—
3.1.6.1	Description	—
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 on 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	Male
3.2.5	Age/weight at study initiation	Age: young adult Average weight: 200 g
3.2.6	Number of animals	5 animals per group.
3.2.7	Control animals	No
3.3	**Administration/ Exposure**	Oral (p.o.), intravenous (i.v.) for whole body autoradiography, intraduodenal (i.d.).
3.3.1	Duration of treatment	Single application
3.3.2	Post-exposure period	Maximum 10 days after oral or intravenous application, 24 hours after intraduodenal application.
3.3.3	Specific activity of test substance	Approx. 60 µCi/mg
3.3.4	**Oral application**	
3.3.4.1	Type	Gavage
3.3.4.2	Concentration	0.1, 5.0, 10.0, or 20.0 mg/kg bw
3.3.4.3	Vehicle	Physiological saline 10 % v/v Cremophor EL solution
3.3.4.4	Concentration in vehicle	0.001; 0.05; 0.1 or 0.2 %
3.3.4.5	Total volume applied	2.0 ml/animal
3.3.4.6	Controls	No
3.3.5	**Intravenous application**	
3.3.5.1	Concentration of test substance	10.0 mg/kg bw

Section A6.2 Toxicokinetics

Annex Point IIA6.2 6.2 Biokinetic behaviour in the rat after single application

3.3.5.2	Vehicle	Physiological saline 5 % v/v Cremophor EL solution
3.3.5.3	Concentration in vehicle	0.1%
3.3.5.4	Total volume applied	2.0 ml/animal
3.3.5.5	Controls	No
3.3.6	**Intraduodenal application**	
3.3.6.1	Concentration of test substance	0.5 mg/kg bw
3.3.6.2	Vehicle	Physiological saline 5 % v/v Cremophor EL solution
3.3.6.3	Concentration in vehicle	0.05%
3.3.6.4	Total volume applied	0.2 ml/animal
3.4	**Examinations**	
3.4.1	Biokinetic parameters	Absorption, distribution, elimination.
3.4.2	Samples	Oral application: urine, faeces, blood, erythrocytes, plasma, bile, organs and tissues (gastrointestinal tract, brain, thyroid, lung, liver, spleen, kidney, adrenal, renal fat, pancreas, muscle, testes, skin, body less gastrointestinal tract), exhaled air, whole body Intravenous application: whole body Intraduodenal application: bile
3.4.3	Sampling time	Oral application: After dosing with 0.1, 5 or 20 mg/kg bw: Gastrointestinal tract (git), body less git,: 48 h Urine: several times during the first two hours, then at 2 h, 4 h, 6 h, 8 h, 10 h, 12 h, 18 h, 24 h, 30 h, 36 h, 42 h, 48 h Faeces: 48 h Only after dosing with 5 mg/kg bw: Blood, erythrocytes, organs and tissues: 2 h, 8 h, 1 d, 2 d, 3 d, 6 d, 10 d. Plasma: 20 min., 40 min., 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, 8 h, 24 h. Urine: several times during the first two days, 3 d, 6 d, 7 d, 8 d, 9 d. Exhaled air: 48 h Only after dosing with 10 mg/kg bw: Whole body: 8 h and 3 d. Intravenous application: whole body: 5 min. Intraduodenal application: within 24 h

Section A6.2 Toxicokinetics

Annex Point IIA6.2 6.2 Biokinetic behaviour in the rat after single application

4 RESULTS AND DISCUSSION

For results see also table A6_2-1.

4.1	**Toxic effects, clinical signs**	Not described.
4.2	**Recovery of labelled compound**	Total recovery was approx. 100% of the administered dose (5 mg/kg bw).
4.3	**Absorption**	From the sum of the activity amounts present in urine, bile and body less gastrointestinal tract after intraduodenal application, plus the amounts of the activity exhaled, an absorption quota of 70 to 80 % was obtained. Maximal relative concentrations of P* = 0.2 (corresponding to equivalent concentrations** of approx. 1 µg/g) were present in plasma only about 1.5 hours after administration of 5 mg/kg. This finding is indicative of a relatively fast rate of absorption. *P = measured radioactivity per gram of tissue/ administered radioactivity per gram bodyweight ** to convert P to dichlofluanid equivalent concentrations [µg/g], the values must be multiplied by the applied dose (mg/kg)
4.4	**Distribution**	The relative concentrations in the body less gastrointestinal tract decreased between 2 hours and 2 days after oral administration of 5 mg/kg by one power of ten to P = 0.021, corresponding to an equivalent concentration C of 0.11 ppm. At 10 days post application, the concentrations present in the body less gastrointestinal tract amounted to only about 0.04 ppm. The majority of the analysed tissues followed this pattern of concentration decline in the range of factor 2. Three to five times higher values were noted transiently in the excretory tissues kidney, liver and adrenal. The relatively highest concentrations of activity were determined in the thyroid. The course of activity concentration in the thyroid differed from that in the other organs. Maximal relative concentrations of P = 1.6 (corresponding to an equivalent concentration of 8 ppm at the 5 mg/kg dose) were not reached in the thyroid until 24 hours after application. During the further course of the study, the activity concentrations present in the thyroid were relatively the highest measured in any tissue. Values of P = 0.54 (≅ 2.7 ppm) were still measured 10 days after application of 5 mg/kg. For a dose of 0.1 mg/kg, the value ought to be dose-proportionally lower, i. e. approximately 0.05 ppm.
4.5	**Elimination**	Orientating tests showed that about 22% of the applied dose was exhaled within 48 hours of oral administration of 5 mg/kg; of this amount more than 90% was expired within the first 8 hours post application. This finding indicates that the radiolabelled fluorodichloromethyl sulfenyl group of the molecule was at least partly split off during metabolism. Following oral administration of the tested doses ranging from 0.1 to 20.0 mg/kg, 40 to 60% of the applied activity was eliminated renally and 20 to 30% was excreted in the faeces within 48 hours. The concentration of activity still present in the body less gastrointestinal tract was between 1.6 – and 2.0%. The slight radiolabelled residues still left in the body less gastrointestinal tract were eliminated more slowly. The slope of the curve indicated an elimination half-life of about one week from day 3 post application.

Section A6.2 Toxicokinetics

Annex Point IIA6.2 6.2 Biokinetic behaviour in the rat after single application

The total radioactivity eliminated was independent of the applied dose, however the ratio of renal elimination to faecal elimination shifted somewhat in favour of faecal elimination at elevated dose levels.

For determination of the extent of the biliary excretion rats were administered 0.5 mg/kg bw by the intraduodenal route, with the result, that fistulated rats excreted only about 7% of the applied dose with the bile.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The biokinetic behaviour of dichlofluanid was examined in rats serving as a model for mammals. For this purpose dichlofluanid was labelled with ^{14}C at the carbon of the fluorodichloromethylmercapto group. To examine the absorption, distribution and elimination of the active substance, [Fluorodichloromethyl-^{14}C]dichlofluanid was administered once to groups of five male Sprague-Dawley rats at dose level of 0.1, 5 or 20 mg/kg bw by the oral route. Radioactivity was measured at different time points in excreta, plasma, and at sacrifice in the animal body and in single tissues. For the latter measurements, additionally groups of male rats were treated with 5 mg/kg bw and sacrificed at different time points. The amount of expired air was determined only after application of 5 mg/kg bw. For whole body autoradiography male rats received a single dose of 10 mg/kg bw by intravenous or oral application. To estimate the extent to which the faecally eliminated amounts of activity were due to already absorbed portions that were then eliminated in the bile, assays were performed on non-anaesthetised, non-fasted rats with fistulated bile ducts for up to 24 hours after intraduodenal administration of 0.5 mg/kg bw. The amounts of activity eliminated in urine, in faeces and in bile and the levels of radiolabelled residues still present in the animal body at the respective times of sacrifice were expressed as percentages of the applied ^{14}C activity. The results relate to the sum of unchanged parent compound and its metabolites.

5.2 Results and discussion

Following oral administration, 70 to 80% of the ^{14}C activity of dichlofluanid was absorbed from the gastrointestinal tract.

The time-courses of activity concentrations in the different organs and tissues, with exception of the thyroid, were not marked by any significant findings. The relative concentrations that peaked at 2 hours post application were mostly well below the uniformly distributed concentration of P = 1. On day 10 post application, the concentrations had declined to values of P≤ 0.005 to 0.002. In the thyroid maximal relative concentrations of P = 1.6 (equivalent to 8 ppm at the 5 mg/kg dose) were not reached in the thyroid until 24 hours after application and in the further course of the study approximately 3 ppm were determined at day10 post-treatment.

Activity was eliminated at a fast rate. The concentration of activity present in the body less gastrointestinal tract was only about 6% of the applied dose at 8 hours after administration of 5 mg/kg, and about 1,6% at 48 hours post application.

In the tested dose range of 0.1 to 20 mg/kg, 40 to 60% of the applied activity was eliminated renally and 20 to 30% was eliminated faecally within 48 hours. The percentage amounts of activity eliminated in the

Section A6.2 Toxicokinetics

Annex Point IIA6.2 6.2 Biokinetic behaviour in the rat after single application

		bile within 24 hours of intraduodenal administration of 0.5 mg/kg. In view of the dose-proportionality of the elimination kinetics in the studied dose range (0.1 to 20 mg/kg), it is to be expected that elimination of activity will be just as fast as described in this study also at still lower dose levels and hence that residues in tissues will then be dose-proportionally lower.
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	17/09/04
Materials and Methods	As described above [IUCLID 5.0 3/4]
Results and discussion	As described above
Conclusion	As described above
Reliability	2
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. [Phenyl-U-^{14}C]dichlofluanid excretion of radioactivity and residues in the body 48 hours after oral application to the rats (values expressed as percentage of the administered radioactivity (mean ± standard deviation, if available))

Parameter	Low dose 0.1 mg/kg bw	Mid dose 5.0 mg/kg bw	High dose 20.0 mg/kg bw
Number of animals examined	4	5	5
$^{14}CO_2$	nd	22	nd
Urine	56 ± 7	54 ± 6	43 ± 3
Faeces	20 ± 3	22 ± 4	33 ± 4
Urine plus faeces	76	76	76
Body excluded git*	2.0 ± 0.2	1.6 ± 0.4	1.8 ± 0.2
Git*	0.6 ± 0.3	0.5 ± 0.2	0.6 ± 0.1
Recovery	78.6	100.1	78.4

*git = gastrointestinal tract

nd = not determined