Section A6.2 Toxicokinetics

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

Official use only

1 REFERENCE

1.1	**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biokinetics part of general metabolism study on rats, [redacted], PF-Report No. [redacted], 1985-07-01 (unpublished)
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 - The substance specification of the non-labelled parent compound is not given (only Batch no.: [redacted])

3 MATERIALS AND METHODS

3.1	**Test material**	
3.1.1	**Non-labelled parent compound**	Dichlofluanid
3.1.2	Lot/Batch number	[redacted]
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**	[Phenyl-UL-^{14}C]dichlofluanid
3.1.5	Lot/Batch number	[redacted]
3.1.5.1	Description	—
3.1.5.2	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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3.1.5.3	Stability	The compound was stable for at least 4 hours (in the application solution) as tested by means of thin-layer-chromatography ([redacted]). The concentration of each administration was checked radiometrically; these measurements served as a reference of the radioactivity in the different biological samples.
3.1.5.4	Radiolabelling	^{14}C-labelled in the benzene ring. [Phenyl-UL-^{14}C]dichlofluanid
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Sprague-Dawley, Mura:SPRA (SPF 68 Han)
3.2.3	Source	[redacted]
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	Age: young adult Weight: About 200 g with exception of the repeated dose experiments. In this case the body weight was about 150 g at the start of the test.
3.2.6	Number of animals	5 animals per sex per group
3.2.7	Control animals	No
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	Single application Repeated application: 15 days One radioactive dose after 14 consecutive non-radioactive doses.
3.3.2	Post-exposure period	Single application: 2 days Repeated application: 2 days
3.3.3	Specific activity of test substance	33.7 µCi/mg (= 1.25 MBq/mg)
3.3.4	Type	Gavage
3.3.5	Concentration of test substance	Single application: High dose level: 20 mg [Phenyl-U-^{14}C]dichlofluanid/kg bw Low dose level: 2 mg [Phenyl-U-^{14}C]dichlofluanid/kg bw Repeated application: Non-radioactive compound: 2 mg/kg bw/day [Phenyl-U-^{14}C]dichlofluanid: 2 mg/kg bw/day
3.3.6	Vehicle	Physiological saline 10 % v/v Cremophor EL solution
3.3.7	Volume applied	Single application: 2.0 ml Repeated application: 10.0 ml/kg bw
3.4	**Examinations**	
3.4.1	Biokinetic parameters	Absorption, distribution, elimination.

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3.3.8	Samples	Urine, faeces, blood (erythrocytes and plasma), organs and tissues (gastrointestinal tract, spleen, liver, kidney, renal fat, femural muscle and bone, testis, heart, brain, thyroid, skin, carcass), exhaled air (both sexes dosed once with 2 mg/kg bw).
3.3.9	Sampling time	Organs and tissues: at sacrifice (2 days post application) Plasma: 0.17, 0.33, 0.67, 1.0 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 24.0, 32.0, 48.0 hours Urine: 2.0, 4.0, 8.0, 24.0, 48.0 hours Faeces: 24.0, 48.0 hours Exhaled air: 0 - 48.0 hours
3.3.10	Further	Organ weights in wet state.

4 RESULTS AND DISCUSSION

4.1	**Toxic effects, clinical signs**	Not described.
4.2	**Recovery of labelled compound**	See also table A6_2-1 in the appendix Single dose application: Males: 96.7 – 97.8% of the administered radioactivity. Females: 93.3 – 99.1% of the administered radioactivity. Repeated dose application: Males: 99.9% of the administered radioactivity. Females: 100.3% of the administered radioactivity.
4.3	**Absorption**	The radioactivity of dichlofluanid labelled in the benzene ring was almost completely absorbed from the gastrointestinal tract following oral administration. The amount of radioactivity excreted in the urine was equivalent to ≥ 90% of the recovered amount, a percentage assumed to represent the experimentally determined absorption quota. The radioactivity of dichlofluanid was absorbed from the rat's gastrointestinal tract at medium to slow speed. The maximum relative concentrations in plasma, reached between 1.5 and 3.0 hours after oral administration, ranged from P^*_{max} = 0.25 to 0.54, also depending from the experimental conditions. To some extent, these figures were slightly dependent on the sex (slower metabolism in the female rat, resulting also in slight quantitative and/or qualitative differences in the metabolism pattern) and on size of the dose (higher relative concentrations in the blood plasma after the low dose). The repeated application of dichlofluanid had only an extremely slight influence on the biokinetic behaviour (a significantly higher maximum relative concentration was measured in plasma of single-dosed females than in the plasma of pre-treated females). For details see table A6_2-2 in the appendix. *P = measured radioactivity per gram of tissue/ administered radioactivity per gram bodyweight

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4.4 Distribution

The relative concentration in the body less gastrointestinal tract, representative of the mean relative concentration in the body, was between P = 0.0030 and 0.0041. These values serve as reference values for the distribution of radioactivity among the individual tissue organs. The relative concentration in plasma, bones, carcass, ovaries and skin were within two times higher or lower than the mean concentration in the body. Slightly elevated concentrations (factor 2 to 3) were measured in spleen and kidney. Distinctly elevated radioactivity concentrations were detectable in liver (factor 3 to 5), thyroid (factor 7 to 12) and erythrocytes (factor 9 to 13) in all rat groups. The elevated concentration in well blood-filled spleen, kidney and liver were considered to have been due at least partially to the elevated residue values in the erythrocyte fraction of the residual blood which remains in the organs also after the so called total exsanguination.

The concentration in brain, muscle and testes were distinctly lower (factor 2) than the mean concentration. The residue concentration in fatty tissue (renal fat) was close to or below the limit of detection in all rat groups.

4.5 Elimination

Radioactivity of dichlofluanid was not excreted in exhaled air.

The total amount of radioactivity eliminated in urine and faeces within two days post application represented approximately 99 % of the recovered amount. The ratio of renal to faecal elimination was distinctly in favour of renal excretion. During the monitored period, about 91 % to 96 % of the recovered amount was eliminated renally, while the percentage of the recovered amount that was excreted in the faeces ranged from 3.1 % to 8.6 % depending on experimental conditions. 50 % of the total amount of renally eliminated activity was excreted in the interval of 5 to 10 hours post application and 90 % in the interval from 20 to 23 hours post application. The very low fecally excreted radioactivity amounts were considered to have been accounted for at least partially by amounts that were absorbed and than eliminated by the biliary or extrabiliary route.

The radiolabelled residues present in the body less gastrointestinal tract (sum of all tissues and organs) two days after application were of very low level in all tested rat groups; their values ranged from 0.28 % to 0.47 % of the recovered amount.

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. Dichlofluanid was labelled with ^{14}C in the benzene ring. For the current investigation the [Phenyl-U-^{14}C]dichlofluanid was used in a set of tests according to OECD-Guideline 417. It was administered orally by gavage as a solution in physiological saline 10 % v/v Cremophor EL once to groups of male and female Sprague-Dawley rats at dose level of 2 or 20 mg/kg bw. Additionally, groups of five male and five female rats received 14 consecutive doses of 2 mg/kg bw non-labelled test substance followed by one radioactive dose of 2 mg/kg.

Radioactivity was measured in plasma and in excreta at different time intervals until study termination (see above in section 3.3.8). Analysis of exhaled air was performed 48 hours post-application. At sacrifice (2 days post-application of the radiolabelled substance), the radioactivity in

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		the animal body and in single tissues was determined. The concentrations of radioactivity measured in plasma and in tissues/organs were expressed as relative concentrations P (= measured radioactivity per gram of tissue or plasma/administered radioactivity per gram bodyweight). The amounts of radioactivity were calculated as percentage of applied radioactivity or, for a better comparison of the results of the individual tests, as percentages of recovered (accountable) radioactivity. The biological material was directly radioassayed, without first being subjected to biochemical extraction procedures. Therefore, the results relate to the sum of unchanged parent compound and its radiolabelled metabolites.
5.2	**Results and discussion**	Dichlofluanid was absorbed completely (≥ 90 %) from the gastrointestinal tract in all dose groups; the absorption kinetics to the point in time at which the radioactivity concentration peaked in plasma (t_{max}) ranged between 1.5 and 3.0 hours, depending from the experimental conditions (sex, dose level, and, in the case of the females, on the pre-treatment). The speed of absorption was slow to medium. Dichlofluanid was found not to accumulate in the carcass or carcass minus gastrointestinal tract. Localisation of activity was confined to the liver, erythrocytes and the thyroids. No differences occurred between the doses. Radioactivity was eliminated rapidly from the body. Within two days post application, about 99 % of the accountable radioactivity were excreted in urine and faeces in all animal groups. Renal excretion was dominant accounting for 91 % to 96 %; 50 % thereof was excreted within the 5 to 10 hours post application and 90 % within 20 to 23 hours post application. Faecal excretion levels varied, depending on sex, between 3.1 % and 8.6 %. Radioactivity of dichlofluanid was not excreted in exhaled air.
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 2/4]
Results and discussion	As described above
Conclusion	As described above
Reliability	2
Acceptability	yes
Remarks	The UK CA agrees with the applicants 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 administration to rats (values in percent of the administered radioactivity (mean ± standard deviation)

Parameter	Low dose 2 mg/kg bw	High dose 20 mg/kg bw	Repeated dose[#] 2 mg/kg bw	Expired air 2 mg/kg bw
Males				
$^{14}CO_2$	—	—	—	< 0.007
Urine	89.2 ± 6.1	90.4 ± 1.6	90.8 ± 4.5	91.8 ± 11.4
Faeces	7.2 ± 4.2	6.9 ± 1.3	8.6 ± 2.8	4.9 ± 0.9
Body excluded git*	0.27 ± 0.03	0.32 ± 0.02	0.32 ± 0.03	0.29 ± 0.02
Git*	0.11 ± 0.04	0.13 ± 0.02***	0.12 ± 0.02	0.53 ± 0.04**
Recovery	96.7 ± 3.5	97.8 ± 0.9	99.9 ± 4.0	97.7 ± 11.3
Females				
$^{14}CO_2$	—	—	—	< 0.001
Urine	89.9 ± 6.3	92.0 ± 2.1	96.4 ± 2.6	93.7 ± 2.7
Faeces	2.9 ± 0.9	4.3 ± 0.9	3.4 ± 0.9	3.3 ± 0.5**
Body excluded git*	0.37 ± 0.01	0.29 ± 0.02	0.35 ± 0.04	0.46 ± 0.03
Git*	0.13 ± 0.06***	0.078 ± 0.015	0.089 ± 0.022	0.72 ± 0.17
Recovery	93.3 ± 5.6	96.7 ± 1.7	100.3 ± 2.9	99.1 ± 1.6

[#] one radioactive dose after 14 consecutive non-radioactive doses

* git = gastrointestinal tract

** n = 3

*** n = 4

Table A6_2-2 [Phenyl-U-^{14}C]dichlofluanid: results of comparison of some biokinetic parameters for both sexes, dose levels or treatment (significance of difference and ratio of means)

Biokinetic Parameters	Low dose 2 mg/kg bw Male/female		Low dose Pre-treatment 2 mg/kg bw Male/female		High dose 20 mg/kg bw Male/female		Low dose/High dose Males		Low dose/High dose Females		Low dose: Single application/ Pre-treatment Males		Low dose: Single application/ Pre-treatment Females	
	Sign.	Ratio	Sign.	Ratio	Sign.	Ratio	Sign.	Ratio	Sign.	Ratio	Sign.	Ratio	Sign.	Ratio
U_{48}	—	0.99	0	0.94	—	0.98	—	0.99	—	0.98	—	0.98	0	0.93
F_{48}	0	2.45	+	2.35	+	1.62	—	1.04	0	0.69	—	0.84	—	0.87
R_{48}	+	0.69	—	0.91	0	1.11	+	0.84	+	1.35	0	0.83	—	1.10
P_{max}	+	0.61	+	0.78	+	0.63	+	1.32	+	1.35	0	1.18	+	1.50
t_{max}	—	0.95	—	0.90	+	0.50	—	1.20	0	0.63	—	1.00	—	0.95
$t_{1/2z}$	—	0.82	—	0.99	0	0.72	—	1.11	—	0.98	—	0.86	—	1.03
AUC	+	0.58	+	0.61	+	0.62	+	0.78	0	0.83	—	1.06	—	1.11
CL	+	1.71	+	1.67	+	1.63	+	1.26	0	1.21	—	0.97	—	0.92
<T>	0	0.93	0	0.87	—	1.09	0	0.88	0	1.03	0	0.85	0	0.80

U_{48}, F_{48}, R_{48}: amounts of radioactivity excreted via Urine or Feces within 48 h or radioactivity labelled Residues in the body excluding the gastrointestinal tract 48 h after administration

P_{max} : Maximum relative plasma concentration

t_{max} : Time interval between administration and the appearance of a maximum concentration in the plasma curve

$t_{1/2z}$: Terminal elimination half-live

AUC: Total area under the curve

CL: Total plasma clearance

<T>: Mean residence time

Sign. Significance of difference (Mann and Whitney):
— : s < 95% 0 : s > 95% + : s > 99%