Section A6.6.4 Genotoxicity in vivo

Annex Point IIA6.6 6.6.4 In vivo cytogenetic study of the bone marrow in Chinese hamsters (cytogenetic in-vivo-test)

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] 1988, KUE 13032 C – Dichlofluanid- In vivo study of the bone marrow in Chinese hamsters to evaluate for a chromosome-damaging effect, [redacted], Report No. [redacted], 1988-03-09 (unpublished)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 the OECD-Guideline 475.
2.2	**GLP**	Yes
2.3	**Deviations**	Yes Deviations of the OECD-Guideline 475: - No determination of the mitotic index was performed.

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2 of dossier.
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2 of dossier.
3.1.2.1	Description	White powder
3.1.2.2	Purity	[redacted] (analytical finding of July 7, 1986) [redacted] (analytical finding of January 13, 1987)
3.1.2.3	Stability	The batch was analysed and approved for at least the duration of the study. The stability in test solvent gave no relevant indication of a change in the active ingredient.
3.1.2.4	Maximum tolerable dose	—
3.2	**Test Animals**	
3.2.1	Species	Chinese hamsters
3.2.2	Strain	Cricetulus griseus
3.2.3	Source	[redacted]
3.2.4	Sex	males and females
3.2.5	Age/weight at study	Age: 8 – 12 weeks

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	initiation	Weight: 26 –35 g
3.2.6	Number of animals per group	5 males + 5 females per dose and sampling time were evaluated. Additional animals were treated for the purpose of replacement in case an animal died in its test group.
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Number of applications	1
3.3.2	Interval between applications	—
3.3.3	Postexposure period	First study: 6, 24, and 48 hours Second study: 24, 32, and 40 hours In both studies the post-exposure period for control groups was 24 hours.
3.3.4	Type	Gavage
3.3.5	Concentration	10,000 mg/kg bw The dose of the test substance was based on a pilot study in which groups of two males and two females had been orally administered 1000 mg/kg, 2500 mg/kg and 5000 mg/kg or 10,000 mg/kg (two groups) dichlofluanid, and had tolerated 1000 mg/kg without symptoms. Symptoms observed at dose levels from 2500 mg/kg were apathy, rough fur, weight loss and somnolence. None of the treated animals died.
3.3.6	Vehicle	0.5 % aqueous Cremophor emulsion
3.3.7	Concentration in vehicle	250 mg/ml
3.3.8	Total volume applied	Dichlofluanid groups: 40 ml/kg bw Negative/positive control: 10 ml/kg bw
3.3.9	Controls	Vehicle (negative control), 30 mg/kg bw cyclophosphamide (positive control) dissolved in demineralised water

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3.4	**Examinations**	
3.4.1	Clinical signs	Yes
3.4.2	Tissue	Bone marrow
	Number of animals:	all animals
	Number of cells:	100 metaphases per animal
	Time points:	First study: 6, 24, and 48 h after treatment Second study: 24, 32, and 40 h after treatment Control groups: 24 h after treatment.
	Type of cells	femoral marrow cells
	Parameters:	numbers and types of structural aberrations
3.5	**Further remarks**	
	4 RESULTS AND DISCUSSION	
4.1	**Clinical signs**	The examined dose was distinctly in the range of acute toxicity. Nine of 39 treated animals died in the first test and 10 of 40 in the second. Distinct signs of illness were also observed. In the first study the following signs were observed: bloody noses, somnolence, prone position and dyspnea. The animals of the second study showed the following signs of illness: apathy, reduced motility, feeble reflexes, distended abdomen, spastic gait, prone position, convulsions, goose stepping, accelerated breathing, spanopea, gummy eyes, and closed palpebral fissures.
4.2	**Haematology / Tissue examination**	—
4.3	**Genotoxicity**	Yes
		10,000 mg/kg bw
4.4	**Other**	—

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5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Dichlofluanid was tested for chromosome-damaging effects using the cytogenetic test on bone marrow, which represents a sensitive in vivo test for chromosome damage in mammals. The methods used in this study were comparable with the OECD-Guideline 475. Existing deviations are described in 2.3 of this section (see above).

The dose of the test substance based on a pilot study in which groups of two males and two females had been orally administered 1000 mg/kg, 2500 mg/kg, and 5000 mg/kg or 10,000 mg/kg dichlofluanid (two groups), and had tolerated 1000 mg/kg without symptoms. Symptoms observed at dose levels from 2500 mg/kg were apathy, rough fur, weight loss and somnolence. None of the treated animals died.

5.2 Results and discussion

The examined dose was distinctly in the range of acute toxicity. Nine of 39 treated animals died in the first test and 10 of 40 in the second. Distinct signs of illness were also observed.

Significant differences between the negative control and the groups treated with 10,000 mg/kg bw dichlofluanid per os were noted in the case of the two parameters significant for assessment of a chromosome-damaging effect (metaphases with aberrations excluding and including gaps). Exchanges were not observed. At a dose of 10,000 mg/kg bw, dichlofluanid thus exerted a mutagenic effect; however, this dose must be termed very high.

In contrast the positive control exerted a distinct chromosome-damaging effect.

5.3 Conclusion

It must be noted, that dichlofluanid exerts mutagenic effects on the bone marrow of the Chinese hamster (i.e. on somatic cells in an in vivo test) following oral administration at a dose of 10,000 mg/kg bw.

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	29/10/04
Materials and Methods	As described above [IUCLID 5.6 2/9]
Results and discussion	As described above
Conclusion	As described above
Reliability	2
Acceptability	Acceptable
Remarks	The UK CA agrees with the applicant's summary and conclusions. However, the UK CA notes that positive findings were only obtained at extremely high, lethal doses.
	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_6_4-2.A Table for cytogenetic in-vivo-test: chromosomal analysis in femoral marrow cells (first study)

		Negative control	10,000 mg/kg bw			Positive control
Sampling time (h)		24	6	24	48	24
Number of metaphases evaluated		1022	1025	1015	1025	1010
Metaphases with aberrations excl. gaps		7	5	18*	24**	233**
(total number, average in percent)		0.68 %	0.49 %	1.77 %	2.34 %	23.07 %
Metaphases with aberrations incl. gaps		19	19	30	36*	255**
(total number, average in percent)		1.86 %	1.85 %	2.96 %	3.51 %	25.25 %
Chromatid aberrations	gaps	12	17	17	16	47
	breaks	5	5	14	22	111
	fragment	—	—	5	2	56
	deletion	—	1	4	1	12
	exchange	—	—	—	—	67
	multiple aberrations without exchanges	2	1	1	—	50
	multiple aberrations with exchanges	**—**	**—**	**—**	**—**	**25**
Additional polyploid metaphases		21	14	6	16	6
(total number, average in percent)		2.05 %	1.37 %	0.59 %	1.56 %	0.59 %

* $p < 0.05$ in chi^2-test

** $p < 0.01$ in chi^2-test

Table A6_6_4-2.B Table for cytogenetic in-vivo-test: chromosomal analysis in femoral marrow cells (second study)

		Negative control	10,000 mg/kg bw			Positive control
Sampling time (h)		24	24	32	40	24
Number of metaphases evaluated		1017	1015	1016	1020	1007
Metaphases with aberrations excl. gaps (total number, average in percent)		9 0.88 %	10 0.99 %	13 1.28 %	26** 2.55 %	224** 22.24 %
Metaphases with aberrations incl. gaps (total number, average in percent)		21 2.06 %	26 2.56 %	44** 4.33 %	51** 5.0 %	268** 26.61 %
Chromatid aberrations	gaps	13	19	35	35	86
	breaks	8	9	13	29	110
	fragment	—	1	—	2	40
	deletion	—	—	1	1	10
	exchange	1	—	—	—	46
	multiple aberrations without exchanges	3	—	—	2	74
	multiple aberrations with exchanges	—	—	—	—	13
Additional polyploid metaphases (total number, average in percent)		7 0.69 %	14 1.38 %	8 0.79 %	6 0.59 %	17* 1.69 %

* $p < 0.05$ in chi^2-test

** $p < 0.01$ in chi^2-test