Section A6.6.5 Genotoxicity in vivo

Annex Point IIA6.6 6.6.5 In-vivo gene mutation assay in foetal cells of mice (Mouse spot test)

Official use only

1 REFERENCE

1.1	**Reference**	[redacted], 1988, KUE 13032 C - Dichlofluanid – Spot test on cross-bred C57B1/6J x T stock mouse foetuses to evaluate for induced somatic changes in the genes of the coat pigment cells, [redacted], Report No. [redacted], 1988-05-31 (unpublished)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 in accordance to the OECD-Guideline 484.
2.2	**GLP**	Yes
2.3	**Deviations**	Yes Compared with the OECD-Guideline 484 the following deviations could be ascertained: - the highest dose used exceed 1 g/kg, - data were not presented on a per litter basis.

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	Fine, white powder
3.1.2.2	Purity	[redacted] (analytical result dated July 15, 1986) [redacted] (analytical result dated January 13, 1987)
3.1.2.3	Stability	The batch used was analytically examined and approved for at least the test period. A stability test in the solvent did not detect an indication of a relevant change in the active ingredient.
3.1.2.4	Maximum tolerable dose	—
3.2	**Test Animals**	
3.2.1	Species	Mouse
3.2.2	Strain	Females: 1. C57B1/6J Bom, 2. C57B1/6J/Ico Males: T stock

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3.2.3	Source	[redacted]
3.2.4	Sex	Males and females
3.2.5	Age/weight at study initiation	Males: Age: 8 –12 weeks Weight: 30 – 45 g Females: Age: 8 –12 weeks Weight: 18 – 41 g
3.2.6	Number of animals per group	As many females with vaginal plugs were treated as were needed to receive at least 300 F1 animals per group for evaluation. 400 mg/kg bw dose group: 198 dams 800 mg/kg bw dose group: 196 dams 1600 mg/kg bw dose group: 340 dams
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Number of applications	1
3.3.2	Application time	Day 10 of gestation
3.3.3	Postexposure period	—
3.3.4	Type	Gavage
3.3.5	Concentration	0, 400, 800 or 1600 mg/kg bw The selection of the Dichlofluanid doses was based on a pilot study in which groups of pregnant females were orally administered 750, 1500, 2500, 5000, or 8000 mg/kg bw. Mortalities occurred from 1500 mg/kg bw and above.
3.3.6	Vehicle	Emulsion of 0.5 % Cremophor in water
3.3.7	Concentration in vehicle	0, 40, 80 or 160 mg/ml
3.3.8	Total volume applied	10 ml/kg bw
3.3.9	Controls	Vehicle
3.3.10	Substance used as Positive Control	40 mg/kg bw 1-ethyl-1-nitrosourea, applied intraperitoneally.

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3.4	**Examinations**	
3.4.1	Clinical signs	Yes
3.4.2	Animals	F1-generation
	Number of animals:	300 animals per treatment group
	Target of examination:	Coloured spots in the coat. These spots are: 1. white mid-ventral spots (WMVS) 2. yellow spots, a result of misdifferentiation (MDS) 3. pigmented and white spots, a result of somatic mutations (RS)
	Number of examinations:	Two
	Time points:	First examination: between day 12 – 16 after birth Second examination: between day 25 - 35 after birth
	Parameters for assessment:	- F1-animals born alive - Litter size - Number of F1-animals without spots - Number of F1-animals with WMVS-spots - Number of F1-animals with RS-spots
3.5	**Further remarks**	—

4 RESULTS AND DISCUSSION

4.1 Clinical signs

Toleration by dams:

The treated mice showed clear symptoms of toxicity at doses of 1600, 800, and 400 mg/kg bw after administration. The following signs were recorded at the day of treatment: apathy, reduced motility, reduced reflexes, roughened fur, emaciation, sunken flanks, staggering gait, wide-legged gait, difficulty in breathing, slow breathing, eyelids stuck together, slitted eyelids and diarrhoea. Later on their behaviour and appetites were normal. Their external appearance and physical activity then remained unaffected.

12 of 198 animals died in the 400 mg/kg dose group, 13 of 196 in the 800 mg/kg dose group and 26 of 340 in the 1600 mg/kg dose group.

Toleration by foetuses:

The litter sizes were not reduced after a single oral treatment of the pregnant dams with Dichlofluanid at doses up to and including 800 mg/kg bw. Application of 1600 mg/kg bw slightly reduced the litter sizes.

4.2 Haematology / Tissue examination —

4.3 Genotoxicity No

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5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Dichlofluanid was evaluated for genetic activity using the mouse spot test, which is regarded as a relevant gene mutation test on the mammal in vivo. The methods in this study used were in accordance with the OECD-Guideline 484.

The selection of the Dichlofluanid doses was based on a pilot study in which groups of pregnant females were orally administered 750, 1500, 2500, 5000, and 8000 mg/kg bw Dichlofluanid. The following symptoms were recorded: apathy, reduced motility, reduced reflexes, roughened fur, staggering gait, prone position, high-stepping gait, difficulty in breathing and eyelids stuck together. In addition, in the 1500 mg/kg dose group two animals died, in the 2500 mg/kg dose group three animals died, in the 5000 mg/kg dose group four animals died and in the 8000 mg/kg dose group six animals died. Based on these findings, the following doses were selected: 1600, 800, and 400 mg/kg bw.

5.2 Results and discussion

After administration of Dichlofluanid the dams showed clear signs of toxicity in all dose groups. Thereafter their external appearance and behaviour were normal. Mortalities were determined in all dose groups.

The litter sizes were not reduced after a single oral treatment of the pregnant dams with Dichlofluanid at doses up to 800 mg/kg bw. Application of 1600 mg/kg bw slightly reduced the litter size.

The results with Dichlofluanid showed that there were no indications of mutagenic effects after a single oral treatment at doses up to 1600 mg/kg bw.

In contrast the positive control induced a clear mutagenic response.

5.3 Conclusion

It may be stated that there were no indications of a mutagenic effect of Dichlofluanid after a single oral treatment at doses up to 1600 mg/kg in the mouse spot test, i.e. in a somatic test system for the detection of induced point mutations in vivo.

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 5.5 7/9]
Results and discussion	As described above
Conclusion	As described above
Reliability	2
Acceptability	Acceptable
Remarks	The UK CA agrees with the applicants and conclusions
	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_4-1. Table for mouse spot test

		Negative control	Low dose 400 mg/kg bw	Mid dose 800 mg/kg bw	High dose 1600 mg/kg bw	Positive control
Number of dams		not reported	198	196	340	not reported
Mortality of the dams		not reported	12	13	26	not reported
F1-animals born alive		449	461	427	576	463
Litter size		7.4 ± 2.1	6.5 ± 2.1	6.7 ± 2.4	5.9 ± 2.4	5.9 ± 2.5
Number of scored F1-animals		418	411	405	546	400
Number of F1-animals: (total number and percent of scored F1-animals)	without spots	408 97.6 %	402 97.8 %	392 96.8 %	524 96.0 %	300 75.0 %
	with WMVS[#] spots	0 0	0 0	2 0.5 %	6* 1.1 %	8** 2.0 %
	with RS[##] spots	10 2.4 %	9 2.2 %	11 2.7 %	16 2.9 %	92** 23.0 %

[#]WMVS: white mid-ventral spots

[##]RS: pigmented and white spots (result of somatic mutations)

*p< 0.05 chi-square test

**p< 0.01 chi-square test