Section A6.6.4 Genotoxicity in vivo

Annex Point IIA6.6 6.6.4 In Vivo Liver UDS

Official use only

1 REFERENCE

1.1	**Reference**	Ex Vivo hepatocyte UDS study with KUE 13032c. Final report [redacted] study No [redacted]).
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	

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Broadly compliant with OECD TG 486.
2.2	**GLP**	Yes
2.3	**Deviations**	The major deviations were the lack of evaluation criteria and the use of only 50 cells per slide. It is noted that 3 slides per animal were used, compared to a minimum of 2 per animal in OECD TG 486.

3 MATERIALS AND METHODS

3.1	**Test material**	
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	
3.1.2.1	Description	White powder
3.1.2.2	Purity	[redacted]
3.1.2.3	Stability	In vehicle: at pH 4 = 15.3 days; at pH 7 = 18.8 hours; at pH 9 = < 10 minutes
3.1.2.4	Maximum tolerable dose	>5000 mg/kg
3.2	**Test Animals**	
3.2.1	Species	Rats
3.2.2	Strain	Wistar strain
3.2.3	Source	[redacted]
3.2.4	Sex	Males
3.2.5	Age/weight at study initiation	Age: NA Weight: approximately 220-225 g
3.2.6	Number of animals per group	3 males per dose.
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral

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3.3.1	Number of applications	1
3.3.2	Interval between applications	NA
3.3.3	Post exposure period	2 hours and 16 hours
3.3.4	Type	Gavage
3.3.5	Concentration	0, 170, 500, 1500 or 4500 mg/kg
3.3.6	Vehicle	0.5 % aqueous Cremophor emulsion
3.3.7	Concentration in vehicle	0, 17, 50, 150 or 450 mg/ml
3.3.8	Total volume applied	All groups: 10 ml/kg
3.3.9	Controls	Vehicle (negative control), 300 mg/kg Methylmethanesulphonate and 100 mg/kg 2-acetylaminofluorene (positive control).
3.4	**Examinations**	
3.4.1	Clinical signs	Assessed
3.4.2	Tissue	Liver
		Number of animals: 3
		Number of cells: 50 cells per slide (three slides per animal)
		Time points: Test substance 2 and 16 h after treatment, Methylmethanesulphonate: 2 h after treatment 2-acetylaminofluorene 16 h after treatment
		Type of cells Heaptocytes
		Parameters: Unscheduled DNA synthesis
3.5	**Further remarks**	—

4 RESULTS AND DISCUSSION

4.1	**Clinical signs**	Clinical signs of toxicity (distress) were observed in 2/3 high dose animals, in the 16-hour exposure group.
4.2	**Haematology / Tissue examination**	
4.3	**Genotoxicity**	No No evidence of UDS was found at either time point. The positive controls gave appropriate responses. It was concluded that dichlofluanid did not cause UDS under the conditions of the study.
4.4	**Other**	Necropsy of the animals in the top-dose group revealed congested lungs, and excessive gas in the intestine and stomach. No evidence of hepatotoxicity was reported.

Trypan blue vital dye call viability tests found viabilities of around 90%.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

5.2 Results and discussion

5.3 Conclusion

5.3.1 Reliability

5.3.2 Deficiencies

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	23/06/05
Materials and Methods	
Results and discussion	No evidence of UDS was found at either time point with the dose range used. The positive controls gave appropriate responses.
Conclusion	Dichlofluanid was not genotoxic under the conditions of the study.
Reliability	2
Acceptability	Acceptable
Remarks	The UK CA has included a robust summary of the above study, as it provides further information on the genotoxic potential of dichlofluanid. The UK CA notes that only 50 cells per slide were counted, from 3 slides per animal. The current OECD TG stipulates a minimum of 100 cells per slide and a minimum of 2 slides per animal. Trypan blue vital dye call viability tests found viabilities of around 90%.
	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 1 Findings for the 2-hour exposure

Dose	Net Nuclear Grain count	% of cells in repair
Vehicle control	-1.83+/-0.27	0.5+/-0.4
170 mg/kg	-1.93+/-0.32	0.6+/-0.5
500 mg/kg	-1.83+/-0.54	0
1500 mg/kg	-1.91+/-0.28	2.4+/-1
4500 mg/kg	-1.29+/-0.22	1+/-1.14
Methylmethanesulphonate	4.14+/-0.56*	42.5+/-5*

* Statistically significantly different from vehicle control

Table 2 Findings for the 16-hour exposure

Dose	Net Nuclear Grain count	% of cells in repair
Vehicle control	-1.8+/-0.15	0.9+/-0.8
170 mg/kg	-1.68+/-0.25	1.8+/-0.8
500 mg/kg	-1.64+/-0.13	1.1+/-0.8
1500 mg/kg	-1.38+/-0.27	1.1+/-0.4
4500 mg/kg	-1.17+/-0.4	0.7+/-0.7
Methylmethanesulphonate	4.40+/-0.51*	46.5+/-5*

* Statistically significantly different from vehicle control