Section A6.6.3 Genotoxicity in vitro

Annex Point IIA6.6 6.6.3 In-vitro gene mutation assay in V79-cells (HPRT-test)

Official use only

1 REFERENCE

1.1	**Reference**	H. Lehn, 1988, KUE 13032 C - Dichlofluanid - Mutagenicity study for the detection of induced forward mutations in the V79-HGPRT assay in vitro, BAYER AG Institute of Toxicology, Report No. 17127, 1988-09-06 (unpublished)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 are in accordance to the OECD-guideline 476.
2.2	**GLP**	Yes
2.3	**Deviations**	Yes Historical controls were not reported.

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 result dated July 14, 1987) [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.
3.2	**Study Type**	In vitro mammalian cell gene mutation test
3.2.1	Organism/cell type	Mammalian cell lines: Chinese hamster lung fibroblasts (V79)
3.2.2	Deficiencies / Proficiencies	—

Section A6.6.3 Genotoxicity in vitro

Annex Point IIA6.6 6.6.3 In-vitro gene mutation assay in V79-cells (HPRT-test)

3.2.3	Metabolic activation system	S9 mix Livers of at least six adult Sprague Dawley rats were used to prepare the S9 mix. For enzyme induction the animals received a single intraperitoneal injection of Aroclor 1254, at dose of 500 mg/kg bw five days before preparation. For preparation, the livers were removed immediately after killing the rats. The livers were homogenised and centrifuged at 9000 x g. Then the supernatant (the S9 fraction) was diluted with a cofactor solution. The amount of S9 fraction in S9 mix is indicated in percent.
3.2.4	Positive control	Without S9 mix: ethylmethane sulfonate (0.9 mg/ml) With S9 mix: 3-methylcholanthrene (5 µg/ml)
3.3	**Application of test substance**	
3.3.1	Concentrations	Without S9 mix: 0, 0.1, 0.15, 0.20, 0.25, 0.30 or 0.35 µg/ml With S9 mix: 0, 5.0, 7.5, 10.0, 12.5, 15.0 or 20.0 µg/ml The test concentrations were based on a pilot study in which dose ranged from 0.06 µg/ml to 1.1 µg/ml without metabolic activation and from 0.098 µg/ml to 25.0 µg/ml with metabolic activation. After determination of the cytotoxicity of dichlofluanid, the concentration range of dichlofluanid for the mutagenicity study was chosen ranging from approx. 0% to 90% reduction in colony forming ability.
3.3.2	Way of application	Dissolved in medium (solvent: DMSO).
3.3.3	Pre-incubation time	—
3.3.4	Other modifications	—
3.4	**Examinations**	
3.4.1	Number of cells evaluated	—
		4 RESULTS AND DISCUSSION
4.1	**Genotoxicity**	
4.1.1	Without metabolic activation	No
4.1.2	With metabolic activation	No
4.2	**Cytotoxicity**	Yes Under both treatment conditions, dichlofluanid induced cytotoxic effects as seen by decreases in relative population growth and cloning efficiency. These results revealed a concentration-related cytotoxicity of dichlofluanid, both with and without S9 mix.

Section A6.6.3 Genotoxicity in vitro

Annex Point IIA6.6 6.6.3 In-vitro gene mutation assay in V79-cells (HPRT-test)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The study was done according to OECD-Guideline 476, though not stated in the study report. The methods used represent further developments of the techniques originally described in the review article of Bradley et al. (Mutation Res. 87: 81 – 142, 1981). The study was carried out with the V79 cell line described by Chu & Malling (Proc. Natl. Acad. Sci. USA 61: 1306 –1312, 1968). The cell line has been shown to be sensitive to a variety of chemical mutagens at its HGPRT gene locus (Bradley et al.; Mutation Res. 87: 81 – 142, 1981). Two duplicate trials were performed each for treatment with and without metabolic activation. The purpose of the test was to assess the ability of dichlofluanid to induce forward mutations at the HPRT locus in V79 cells.
5.2	**Results and discussion**	Under both treatment conditions, cytotoxicity was induced. There were no increases in mutant frequency which were significantly elevated over the negative controls. In contrast, the positive controls ethylmethanesulfonate (without S9 mix) and 3-methylcholanthrene (with S9 mix) revealed a clear mutagenic effect in the assay.
5.3	**Conclusion**	The test substance can be considered as non-mutagenic in the V79-HPRT assay, both with and without metabolic activation.
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	28/10/04
Materials and Methods	As described above [IUCLID 10/12]
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 sufficiently high concentrations may not have been used.
	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_1-1.A Table for gene mutation assay: V79-HPRT-test

	Treatment without S9 mix			
Concentration [µg/ml]	**Mutant Frequency#** (Thioguanin-resistant mutants per 10^6 clonable cells)			
	1st trial with duplicates		2nd trial with duplicates	
Negative control	4.0	1.7	2.3	2.9
Vehicle control	5.5	1.9	4.0	4.4
0.10	1.0	0.7	2.1	2.9
0.15	2.3	1.6	3.7	1.8
0.20	1.1	0.9	5.4	4.3
0.25	1.9	1.6	6.5	7.5
0.30	6.0	—	5.2	3.0
0.35	2.6	1.7	4.4	4.0
Positive control	656.7*	1131.3*	353.2*	302.0*

* = significant increase, $p < 0.05$

Table A6_6_1-1.B Table for gene mutation assay: V79-HPRT-test

	Treatment with S9 mix			
Concentration [µg/ml]	**Mutant Frequency#** (Thioguanin-resistant mutants per 10^6 clonable cells)			
	1st trial with duplicates		2nd trial with duplicates	
Negative control	3.7	6.2	2.3	1.3
Vehicle control	2.7	0.6	1.6	1.2
5.0	7.1	3.0	1.7	1.6
7.5	6.7	4.1	2.0	1.5
10.0	3.2	4.8	0.8	1.4
12.5	3.1	4.2	1.4	1.1
15.0	2.6	4.6	1.9	1.4
20.0	1.4	2.5	1.1	1.8
Positive control	53.3*	34.4*	43.5*	40.4*

* = significant increase, $p < 0.05$

Mutant frequency = Total number of mutant colonies x 100/total number of cells seeded x absolute cloning efficiency

Table A6_6_1-1.C Table for Gene Mutation Assay: V79-HPRT-test

Treatment without S9 mix		
Concentration [µg/ml]	**Relative Survival*** (% of vehicle control)	
	1st trial with duplicates	2nd trial with duplicates
Negative control	73.4	90.5
Vehicle control	100.0	100.0
0.10	53.3	77.0
0.15	72.1	89.3
0.20	65.8	89.3
0.25	70.7	96.2
0.30	70.4	80.7
0.35	82.7	66.3
Positive control	60.7	59.6

Table A6_6_1-1.D Table for Gene Mutation Assay: V79-HPRT-test

Treatment without S9 mix		
Concentration [µg/ml]	**Relative Survival*** (% of vehicle control)	
	1st trial with duplicates	2nd trial with duplicates
Negative control	90.5	90.2
Vehicle control	100.0	100.0
5.0	97.9	85.3
7.5	86.2	95.1
10.0	93.8	102.7
12.5	79.5	61.4
15.0	75.3	85.9
20.0	53.3	88.0
Positive control	83.4	89.1

* Relative survival = Mean number of colonies (treated cultures) × 100/ mean number of colonies (vehicle control cultures)

Table A6_6_1-1.E Table for Gene Mutation Assay: V79-HPRT-test

Treatment without S9 mix				
Concentration [µg/ml]	**Relative Population Growth**** (% of vehicle control)			
	1st trial with duplicates		2nd trial with duplicates	
Negative control	62.7	54.4	119.2	92.9
Vehicle control	100.0	100.0	100.0	100.0
0.10	64.1	50.7	105.1	95.7
0.15	48.1	68.7	120.0	145.5
0.20	66.1	73.0	104.2	99.4
0.25	74.7	62.2	72.9	55.4
0.30	64.1	61.9	84.8	80.6
0.35	57.7	54.5	74.6	64.4
Positive control	65.1	60.7	55.0	58.5

Table A6_6_1-1.F Table for Gene Mutation Assay: V79-HPRT-test

Treatment with S9 mix				
Concentration [µg/ml]	**Relative Population Growth**** (% of vehicle control)			
	1st trial with duplicates		2nd trial with duplicates	
Negative control	159.7	140.3	86.1	102.1
Vehicle control	100.0	100.0	100.0	100.0
5.0	72.3	91.9	84.5	110.9
7.5	99.1	95.4	79.5	104.9
10.0	75.3	101.3	110.4	131.3
12.5	116.3	102.5	80.0	77.1
15.0	73.8	69.7	93.9	67.9
20.0	47.7	74.0	107.8	81.0
Positive control	152.8	97.0	60.4	69.9

**Relative population growth (%) = (Treated culture population increase over the expression period/vehicle control population increase over the expression period) × 100

This parameter shows the cumulative growth of the treated cell populations, relative to the vehicle control, over the expression period and prior to mutant selection. Values of less than 100% indicate growth inhibition as result of toxicity of the test substance.