Section A6.6.3 Genotoxicity in vitro

Annex Point IIA6.6 6.6.3 In-vitro gene mutation assay in L5178Y-cells ($TK^{+/-}$ test)

Official use only

		1 REFERENCE
1.1	**Reference**	M. A. Cifone, 1985, Mutagenicity evaluation of KUE 13032 C (VM) - c n. Dichlofluanid – in the mouse lymphoma forward mutations assay, Litton Bionetics, Inc., Department of Molecular Toxicology, Report No. 3327, 1985-06-13 (unpublished)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 comparable with the OECD-guideline 476.
2.2	**GLP**	Yes
2.3	**Deviations**	Yes In deviation of the OECD-Guideline 476 no colony sizing were performed. Historical controls were not documented.
		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]
3.1.2.3	Stability	The batch used was analytically examined and approved for at least the test period. Dichlofluanid is stable in DMSO at room temperature up to 4 hours.
3.2	**Study Type**	In vitro mammalian cell gene mutation test
3.2.1	Organism/cell type	Mammalian cell lines: Mouse lymphoma L5178Y cells
3.2.2	Deficiencies / Proficiencies	—
3.2.3	Metabolic activation system	S9 mix S9 homogenate was commercially prepared and consisted of the 9000 × g supernatant prepared from Aroclor 1254-induced adult male rat livers.
3.2.4	Positive control	Without S9 mix: ethylmethane sulfonate (0.25 to 0.5 µl/ml) With S9 mix: 3-methylcholanthrene (1.0 to 4.0 µg/ml)

Section A6.6.3 Genotoxicity in vitro

Annex Point IIA6.6 6.6.3 In-vitro gene mutation assay in L5178Y-cells ($TK^{+/-}$ test)

3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	Without S9 mix: First assay: 0, 0.1, 0.2, 0.25, 0.3, 0.4 or 0.5 µg/ml Second assay: 0, 0.1, 0.5, 0.6, 0.8, 1.0 or 1.2 µg/ml Third assay: 0, 0.3, 0.4, 0.5, 0.6, 0.8 or 1.0 µg/ml With S9 mix: First assay: 0, 1.0, 4.0, 6.0, 8.0, 10.0 or 12.0 µg/ml Second assay: 0, 4.0, 8.0, 10.0, 12.0, 14.0 or 16.0 µg/ml The test concentrations were based on a pilot study. The concentration range of dichlofluanid for the mutagenicity study was chosen ranging from approx. 10% to 100% 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	Yes Second assay: 0.6, 0.8, 1.0, and 1.2 µg/ml Third assay: 1.0 µg/ml
4.1.2	with metabolic activation	Yes First assay: 6.0, 8.0, 10.0, and 12.0 µg/ml Second assay: 10.0, 12.0, 14.0, and 16.0 µg/ml
4.2	**Cytotoxicity**	Yes Treatments up to 1.2 µg/ml without activation induced a wide range of toxicity (7.6 % to 81.2 % relative growth). Treatments up to 16 µg/ml with activation induced decreases in relative population growth between 13.7 % and 77.9 % survival.

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5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The study was performed according to OECD-Guideline 476, with some deviations as described in 2.3. Under non-activation conditions three assays were performed in duplicates. Under metabolic conditions two trials were performed employing duplicates. The purpose of the test was to assess the ability of dichlofluanid to induce forward mutations at the TK locus in mouse lymphoma L5178Y cells.
5.2	**Results and discussion**	Treatments up to 1.2 µg/ml without activation and up to 16 µg/ml with activation were assayed and a wide range of toxicity was induced. Small but significant increases in the mutant frequency were induced at moderate to high toxicity. The increase ranged from 1.8-fold to 3.3-fold above the background mutant frequency (average of solvent controls).
5.3	**Conclusion**	The test substance is therefore considered weakly active in the Mouse Lymphoma Forward Mutation 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	09/08/06
Materials and Methods	As described above [IUCLID 5.5 11/12]. The UK CA notes that no information was available on colony sizing, but this is not considered to have compromised the interpretation of the study.
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.
	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

Treatment without S9 mix								
Mutant Frequency[#] (Trifluorothymidine-resistant mutants per 10^6 clonable cells)								
Concen-tration [µg/ml]	**1st trial**		**Concen-tration [µg/ml]**	**2nd trial**		**Concen-tration [µg/ml]**	**3rd trial**	
Vehicle control	27.6	26.8	**Vehicle control**	29.1	30.9	**Vehicle control**	26.2	29.2
	36.5	32.8		25.0	20.5		27.1	29.5
0.10	31.3	20.8	**0.10**	23.0	30.1	**0.30**	50.3	26.6
0.20	41.7	29.4	**0.50**	28.4	40.0	**0.40**	34.2	37.4
0.25	29.4	21.2	**0.60**	51.0*	47.8	**0.50**	35.3	28.6
0.30	19.1	18.6	**0.80**	49.3	66.8*	**0.60**	36.6	—
0.40	33.3	32.3	**1.00**	85.4*	80.3*	**0.80**	42.6	44.0
0.50	34.4	29.8	**1.20**	74.3*	—	**1.00**	62.6*	—
Positive control 0.25 µl/ml	511.2*	445.7*	**Positive control 0.25 µl/ml**	135.6*	127.9*	**Positive control 0.25 µl/ml**	527.7*	502.3*
Positive control 0.40 µl/ml	717.5*	758.8*	**Positive control 0.40 µl/ml**	1098.3*	777.0*	**Positive control 0.40 µl/ml**	834.8*	969.7*

[#]Mutant frequency = Total mutant colonies/total viable colonies × 2 × 10^{-4}

*Mutant frequency exceeded the minimum criterion.

The minimum criterion is defined as the mutant frequency that is at least 150 % of the concurrent background frequency plus 10 × 10^{-6}.

Table A6_6_1-1.B Table for gene mutation assay

Treatment with S9 mix					
Mutant Frequency[#] (Trifluorothymidine-resistant mutants per 10^6 clonable cells)					
Concentration [µg/ml]	**1st trial**		**Concentration [µg/ml]**	**2nd trial**	
Vehicle control	57.0	51.8	**Vehicle control**	48.6	42.5
	59.8	76.1		41.1	56.9
1.0	85.6	78.4	**4.0**	58.7	51.0
4.0	97.8	83.2	**8.0**	72.2	69.1
6.0	102.9*	81.8	**10.0**	—	102.4*
8.0	109.1*	102.2*	**12.0**	85.3*	100.6*
10.0	144.1*	88.1	**14.0**	120.2*	91.4*
12.0	140.1*	150.3*	**16.0**	96.3*	154.1*
Positive control 2.5 µg/ml	249.7*	249.5*	**Positive control 2.5 µg/ml**	218.6*	209.8*
Positive control 4.0 µg/ml	407.2*	457.0*	**Positive control 4.0 µg/ml**	254.1*	315.4*

[#]Mutant frequency = Total mutant colonies/total viable colonies × 2×10^{-4}

*Mutant frequency exceeded the minimum criterion.

The minimum criterion is defined as the mutant frequency that is at least 150 % of the concurrent background frequency plus 10×10^{-6}.

Table A6_6_1-1.C Table for gene mutation assay

Treatment without S9 mix								
Relative Growth (%) (relative suspension growth × relative cloning efficiency/100)								
Concen-tration [µg/ml]	**1st trial**		**Concen-tration [µg/ml]**	**2nd trial**		**Concen-tration [µg/ml]**	**3rd trial**	
Vehicle control	100	100	**Vehicle control**	100	100	**Vehicle control**	100	100
	100	100		100	100		100	100
0.10	73.2	79.4	**0.10**	58.8	65.9	**0.30**	65.6	57.6
0.20	81.2	76.1	**0.50**	35.0	31.8	**0.40**	54.4	49.3
0.25	60.2	63.5	**0.60**	21.0	25.8	**0.50**	55.3	51.5
0.30	40.7	53.5	**0.80**	16.0	11.2	**0.60**	49.9	—
0.40	40.0	26.3	**1.00**	9.7	8.7	**0.80**	31.7	44.7
0.50	25.2	43.2	**1.20**	7.6	—	**1.00**	19.1	—
Positive control 0.25 µl/ml	52.5	62.0	**Positive control 0.25 µl/ml**	62.1	88.3	**Positive control 0.25 µl/ml**	48.1	45.8
Positive control 0.40 µl/ml	35.8	32.0	**Positive control 0.40 µl/ml**	17.5	23.8	**Positive control 0.40 µl/ml**	26.0	22.1

Table A6_6_1-1.D Table for gene mutation assay

Treatment with S9 mix					
Relative Growth (%) (relative suspension growth × relative cloning efficiency/100)					
Concentration [µg/ml]	**1st trial**		**Concentration [µg/ml]**	**2nd trial**	
Vehicle control	100	100	**Vehicle control**	100	100
	100	100		100	100
1.0	77.4	77.9	**4.0**	61.0	44.6
4.0	47.0	61.0	**8.0**	32.0	40.5
6.0	52.2	50.8	**10.0**	18.6	18.5
8.0	35.2	46.5	**12.0**	20.7	16.3
10.0	24.3	41.1	**14.0**	23.1	15.8
12.0	23.6	13.7	**16.0**	23.7	15.0
Positive control 2.5 µg/ml	60.9	65.5	**Positive control 2.5 µg/ml**	51.2	49.9
Positive control 4.0 µg/ml	44.0	30.6	**Positive control 4.0 µg/ml**	35.3	29.7