Section A6.6.1 Genotoxicity in vitro

Annex Point IIA6.6 6.6.1 In-vitro gene mutation study in bacteria (Salmonella typhimurium-reverse mutation assay)

Official use only

1 REFERENCE

1.1	**Reference**	B. Herbold, 1984, KUE 13032 C – Dichlofluanid - Salmonella/microsome test to evaluate for potential point mutation, BAYER AG Institute of Toxicology, Report No. 12834, 1984-08-06 (unpublished)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 in this study are comparable to OECD-Guideline 471.
2.2	**GLP**	No GLP was not compulsory at the time the study was performed.
2.3	**Deviations**	Yes The following deviations in comparison to the OECD-Guideline 471 occurred: - The Salmonella typhimurium strain TA 102 or E. coli WP2 strains not used (TA 102 or E. coli WP2 strains have an AT base pair at the primary reversion site to detect certain oxidising mutagens, cross-linking agents and hydrazines). - As described in the OECD-Guideline 471, equivocal results should be clarified by further testing preferably using a modification of experimental conditions. In this study the same experimental procedure was performed for the first test as well as for the repeat test. - 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	—
3.1.2	Specification	As given in section 2 of dossier.
3.1.2.1	Description	—
3.1.2.2	Purity	[redacted] active substance
3.1.2.3	Stability	The stability test in the solvent did not reveal any relevant indication of a change in dichlofluanid.
3.2	**Study Type**	Bacterial reverse mutation test

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3.2.1	Organism/cell type	S. typhimurium: TA 1535, TA 1537, TA 98, TA 100
3.2.2	Deficiencies / Proficiencies	—
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	With and without S9 mix: Endoxan (145 µg/plate for TA 1535 and 290 µg/plate for TA 100; equivalent to 100 µg and 200 µg cyclophosphamide, respectively), trypaflavine (50 µg/plate for TA 1537 and TA 98), 2-Aminoanthracene (3 µg/plate for all strains)
3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	First test: With and without S9 mix: 0, 25, 50, 100, 200, 400, 800 µg/plate Repeat test: With S9 mix: 0, 3, 6, 12, 24, 48 µg/plate Without S9 mix: 0, 19, 38, 76, 152, 304 µg/plate
3.3.2	Way of application	Dissolved in medium, the solvent for dichlofluanid, trypaflavine and 2-amino-anthracene was DMSO, and demineralised water for endoxan.
3.3.3	Pre-incubation time	—
3.3.4	Other modifications	—
3.4	**Examinations**	See tables in appendix for examinations and results. First and repeat test: with and without metabolic activation (30% S9 mix) Additional to the genotoxicity the following parameters of bacteriotoxicity were determined: 1. Gross appraisal of background growth on the plates for mutant determination. 2. Marked and dose-dependent reduction in the mutant count per plate compared to the negative control. 3. Titre determination.
3.4.1	Number of cells evaluated	—

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4 RESULTS AND DISCUSSION

4.1	**Genotoxicity**	
4.1.1	Without metabolic activation	Yes Repeat test: TA 98: 3, 6, 12, 24 µg/plate
4.1.2	With metabolic activation	Yes First test: TA 98: 25, 50, 100, 200 µg/plate TA 100: 100, 200 µg/plate Repeat test: TA 98: 76, 152, 304 µg/plate TA 100: 152, 304 µg/plate
4.2	**Cytotoxicity**	Yes Dichlofluanid produced bacteriatoxic effects from 25 µg per plate onward, both with and without S-9 mix. Lower doses (up to and including 19 µg per plate) did not induce cytotocity.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The mutagenicity of the test substance was evaluated with the Salmonella/microsome test, also termed the Ames Test, as described by Ames et al. (Proc. Nat. Acad. Sci. 70: 2281-2285, 1973 and Mutation Res. 31: 347-364, 1975) and Maron and Ames (Mutation Res. 113: 173-215, 1983. The study was done according to the OECD-Guideline 471 with slight deviations as described in 2.3 this section (see above).
5.2	**Results and discussion**	A biologically relevant increase in mutant counts to slightly more than double those of the respective negative control was noted for Salmonella typhimurium TA 100 and TA 98. The lowest effective dose at which this finding could be reproduced was 152 µg/plate for TA 100, and 76 µg/plate for TA 98.
5.3	**Conclusion**	Dichlofluanid must therefore be considered clearly mutagenic in the Salmonella/microsome test.
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 [5.5 1/12]
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.
	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: first test for TA 1535, TA 100, TA 1537, TA 98

Concentration [µg/plate]	**Number of mutant cells** (mean ± standard deviation)							
	TA 1535		**TA 100**		**TA 1537**		**TA 98**	
	-S9	**+S9 (30%)**	**-S9**	**+S9 (30%)**	**-S9**	**+S9 (30%)**	**-S9**	**+S9 (30%)**
0	19 ± 3	12 ± 4	73 ± 11	95 ± 17	7 ± 3	7 ± 1	12 ±3	19 ± 5
25	11 ± 2	16 ± 3	65 ± 7	103 ± 11	*b***	8 ± 3	16 ± 3	40# ± 3
50	*b***	21 ± 7	35** ± 5	125 ± 17	0**	9 ± 3	*b***	51# ± 12
100	0**	18 ± 2	*b***	167# ** ± 26	0**	9 ± 2	0**	61# ± 4
200	0**	17** ± 4	0**	174# ** ± 46	0**	16** ± 3	0**	69# ± 9
400	0**	*b***	0**	60** ± 24	0**	*b***	0**	9** ± 3
800	0**	0**	0**	0**	0**	0**	0**	0**
Positive control	31 ± 7	343# ± 36	71 ± 6	415# ± 81	29# ± 6	364# ± 59	47# ± 19	1474# ± 202
2-Amino-antharacene	22 ± 4	317# ± 24	93 ± 3	1311# ± 36	8 ± 4	88# ± 3	27# ± 5	812# ± 254

** bacteriotoxic effect

b background

mutagen

Table A6_6_1-1.B: Table for gene mutation assay: repeated test for TA 1535, TA 100, TA 1537, TA 98

Concentration [µg/plate]		**Number of mutant cells** (mean ± standard deviation)							
		TA 1535		**TA 100**		**TA 1537**		**TA 98**	
-S9	**+S9**	**-S9**	**+S9 (30%)**	**-S9**	**+S9 (30%)**	**-S9**	**+S9 (30%)**	**-S9**	**+S9 (30%)**
0	**0**	30 ± 4	17 ± 1	73 ± 13	133 ± 12	5 ± 3	13 ± 3	17 ± 6	28 ± 3
3	**19**	23 ± 6	22 ± 4	128 ± 19	151 ± 16	7 ± 2	11 ± 5	34# ± 6	35 ± 8
6	**38**	20 ± 5	21 ± 7	142 ± 7	163 ± 4	9 ± 2	10 ± 4	43# ± 11	44 ± 6
12	**76**	21 ± 6	21 ± 6	135 ± 18	216** ± 12	11 ± 4	8 ± 3	78# ± 6	65# ± 18
24	**152**	16**± 3	30** ± 6	114 ± 14	261# ** ± 21	2**± 2	7 ± 4	67# ± 10	73# ± 15
48	**304**	7** ± 3	24** ± 21	59**± 4	275# ** ± 27	*b*	10** ± 4	*b***	79# ± 19
Positive control		19 ± 5	382# ± 26	94 ± 9	356# ± 116	45# ± 17	354# ± 18	56# ± 12	1441# ± 271
2-Amino-antharacene		24 ± 5	259# ± 12	120 ± 13	1414# ± 142	14# ± 1	59# ± 13	36# ± 12	385# ± 66

** bacteriatoxic effect

b background

mutagen