Section A6.6.2 Genotoxicity in vitro

Annex Point IIA6.6 6.6.2 In-vitro cytogenicity study in human lymphocytes

Official use only

1 REFERENCE

1.1	**Reference**	B. Herbold, 1986, KUE 13032 C - Dichlofluanid - In vitro cytogenetic study on human lymphocyte cultures to evaluate for chromosome-damaging effects, BAYER AG Institute of Toxicology, Report No. 14707, 1986-06-16 (unpublished)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 the study are comparable to OECD-Guideline 473.
2.2	**GLP**	Yes
2.3	**Deviations**	Yes - The mitotic index was defined as mitoses in % of the negative control and not as the ratio of cells in metaphase divided by the total number of cells observed in a population of cells. - 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	—
3.1.2.2	Purity	[redacted]
3.1.2.3	Stability	The batch used was analysed and approved for at least the duration of the study. The stability test in solvent gave no relevant indication of a change in the active ingredient.
3.2	**Study Type**	In vitro mammalian chromosome aberration test
3.2.1	Organism/cell type	Primary cultures: human lymphocytes

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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	Without S9 mix: mitomycin C (0.1 µg/ml) With S9 mix: cyclophosphamide (10.0 µg/ml)
3.3	**Application of test substance**	
3.3.1	Concentrations	With/without S9 mix: 0, 1, 3, 10 µg/ml culture medium. The levels used were based on a pilot test in which the concentrations were 100, 30, 10, 3, and 1 µg/ml. A level of 10 µg/ml was thus established as the maximum concentration for the main study.
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	About 200 metaphase cells per concentration with and without S9 mix were analysed for structural chromosome aberrations. The mitotic index was determined by counting 4000 cells per concentration.

4 RESULTS AND DISCUSSION

4.1	**Genotoxicity**	
4.1.1	Without metabolic activation	Yes 3 and 10 µg/ml
4.1.2	With metabolic activation	Yes 10 µg/ml
4.2	**Cytotoxicity**	Yes With and without S9 mix: in the treated cultures, the mitotic rate underwent a concentration-related reduction relative to the negative control at levels of 3 µg/ml and above.

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5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The study was done according to OECD-Guideline 473, though not stated in the study report. The methods used represent further development of cultivation and preparation techniques originally described by Moorhead et al. (Exp. Cell Res. 20, 613-616, 1960)

The study was carried out on human lymphocyte cultures in vitro. Human lymphocytes were gained from the blood of one male and one female donor. There were two cultures per donor per concentration.

These cultures of peripheral blood from healthy test persons allow testing of the potential chromosome-damaging effect of a substance on human cells. In addition, the cell division rate and thus the cytotoxic effect of the test substance may be evaluated by determination of the mitotic index.

5.2 Results and discussion

The results of the mitotic index determination demonstrate a significant concentration-related cytotoxic effect by dichlofluanid both with and without S9 mix.

Treatment-related variations in parameters significant for evaluation of a clastogenic effect (metaphases with aberrations including/excluding gaps and metaphases with exchanges) were observed between the negative control and the groups treated with dichlofluanid in vitro at levels from 3 µg/ml without S9 mix and from 10 µg/ml with S9 mix.

The positive control cyclophosphamide exerted definite clastogenic action, thus demonstrating the sensitivity of the system to chromosome-damaging agents. No relevant effect could be observed with the second positive control mitomycin C.

5.3 Conclusion

It may be stated that dichlofluanid demonstrated a chromosome-damaging effect in the cytogenetic in vitro test on human lymphocyte cultures in the cytotoxic range at concentrations up to 10 µg/ml both with and without S9 mix.

5.3.1 Reliability 1

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 5.4 8/12]
Results and discussion	As described above
Conclusion	As described above
Reliability	1
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_1-2.A: Table for Cytogenetic In-Vitro-Test: Chromosomal Analysis

		Treatment without S9 mix				
		Negative control	**Low dose 1 µg/ml**	**Mid dose 3 µg/ml**	**High dose 10 µg/ml**	**Positive control**
Cytotoxicity (mitoses)		no	no	yes	yes	no
Mitoses absolute		91	77	50*	9**	77
and in % of negative control		100 %	84.6 %	55.0 %	9.9 %	84.6 %
Chromatid aberrations	gaps	1	—	3	4	—
	breaks	2	9	10	28	7
	fragments	1	—	1	3	—
	deletion	—	—	—	2	—
	exchange	1	—	1	1	—
	multiple aberrations	—	—	1	6	—
Polyploidy		1	4	8[#]	7[#]	3

[#] $p \leq 0.05$ in chi-square test

*$p \leq 0.01$ in chi-square test

** $p \leq 0.001$ in chi-square test

Table A6_6_1-2.B: Table for Cytogenetic In-Vitro-Test: Chromosomal Analysis

		Treatment with S9 mix				
		Negative control	**Low dose 1 µg/ml**	**Mid dose 3 µg/ml**	**High dose 10 µg/ml**	**Positive control**
Cytotoxicity (mitoses)		no	no	yes	yes	no
Mitoses absolute		66	60	28**	9**	69
and in % of negative control		100 %	90.9 %	42.4 %	13.6 %	104.6 %
Chromatid aberrations	gaps	2	2	1	5	5
	breaks	5	8	14	21	21
	fragments	—	—	3	4	2
	deletion	—	—	—	—	1
	exchange	—	—	1	1	2
	multiple aberrations	—	—	—	—	—
Polyploidy		1	2	4	0	1

** $p \leq 0.001$ in chi-square test