Section A6.6.4 Genotoxicity in vivo

Annex Point IIA6.6 6.6.4 Micronucleus test on the male mouse

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted] (2004): Dichlofluanid techn. Micronucleus-Test on the male mouse [redacted] Report No. [redacted], 2004-12-16 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	LANXESS Deutschland GmbH
1.2.2	Companies with letter of access	
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**	Yes OECD 474, (July 1997) EC Method B.12 (May 2000)
2.2	**GLP**	Yes
2.3	**Deviations**	No
		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	Fine white powder
3.1.2.2	Purity	---
3.1.2.3	Stability	Until December 15, 2004
3.1.2.4	Maximum tolerable dose	60 mg/kg bw
3.2	**Test Animals**	
3.2.1	Species	Mouse
3.2.2	Strain	Hsd/Win: NMRI
3.2.3	Source	[redacted]
3.2.4	Sex	Males
3.2.5	Age/weight at study initiation	Age: 6 – 12 weeks Weight: 37 –44 g
3.2.6	Number of animals per group	5 males per dose 5 additional males for a 60 mg/kg replacement group
3.2.7	Control animals	Yes

Section A6.6.4 Genotoxicity in vivo

Annex Point IIA6.6 6.6.4 Micronucleus test on the male mouse

3.3	**Administration/ Exposure**	Intraperitoneal
3.3.1	Number of applications	Negative control and treatment groups: 2 Positive control: 1
3.3.2	Interval between applications	24 h
3.3.3	Post-exposure period	24 h after last treatment
3.3.4	Type	Intraperitoneal injection
3.3.5	Concentration in vehicle	No data
3.3.6	Vehicle	Test substance was suspended in 0.5 % Cremophor emulsion.
3.3.7	Total volume applied	10 mL/kg b.w.
3.3.8	Dose applied	15, 30 and 60 mg/kg b.w.
3.3.9	Controls	Vehicle (negative control)
3.3.10	Substance used as positive control	Cyclophosphamide (monohydrdate) 1 × 20 mg/kg bw
3.4	**Examinations**	
3.4.1	Clinical signs	Yes
3.4.2	Tissue	Bone marrow
		Number of animals: all (30 animals)
		Number of cells: 2000 evaluated polychromatic erythrocytes per animal
		Time points: 24 h after last treatment
		Type of cells erythrocytes in bone marrow
		Parameters: polychromatic/normochromatic erythrocytes ratio
3.5	**Further remarks**	—

4 RESULTS AND DISCUSSION

4.1	**Clinical signs**	Treated animals showed the following compound-related symptoms until sacrifice: apathy, roughened fur, loss of weight, spasm, twitching, periodically stretching of body, difficulty in breathing, slitted eyes, closed eyes and reduced body temperature. One animal died in the 30 mg/kg dose group, four animals died in the 60 mg/kg dose group. No symptoms or deaths were recorded in the control groups.
4.2	**Haematology**	See Table 6_6_4-1 in appendix
4.3	**Genotoxicity**	No
4.4	**Other**	—

Section A6.6.4 Genotoxicity in vivo

Annex Point IIA6.6 6.6.4 Micronucleus test on the male mouse

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The micronucleus test was employed to investigate Dichlofluanid techn. in male NMRI mice for a possible clastogenic effect on the chromosomes of bone-marrow erythroblasts. The known clastogen and cytostatic agent, cyclophosphamide, served as positive control.

Male mice were treated with two intraperitoneal doses of 15, 30 and 60 mg/kg b.w., respectively, at 24 h intervals. Males of the positive control received a single intraperitoneal administration of 20 mg/kg b.w. cyclophosphamide. The femoral marrow of all groups was prepared 24 h after the last administration.

5.2 Results and discussion

Male mice treated twice with Dichlofluanid techn. in doses up to 60 mg/kg showed symptoms of toxicity after administration, starting at 15 mg/kg. These symptoms demonstrate relevant systemic exposure of males to technical Dichlofluanid. One of five animals in the 30 mg/kg group and four of ten animals in the 60 mg/kg group died before the end of the test due to the acute intraperitoneal toxicity of Dichlofluanid techn.. There was an altered ratio between polychromatic and normochromatic erythrocytes. This finding demonstrates relevant systemic exposure of the males to Dichlofluanid techn..

No indicationsof a clastogenic effect of technical Dichlofluaniod were found in any dose group.

The positive control, cyclophosphamide, had a clear clastogenic effect, as is shown by the biologically relevant increase in polychromatic erythrocytes with micronuclei. The ratio of polychromatic to normochromatic erythrocytes was not altered.

5.3 Conclusion

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	5/06/05
Materials and Methods	As described above
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_4-1.A Table for micronucleus test in vivo (first test)

Two treatments at 24 h intervals		**Negative control** (vehicle)	**Dose 1**	**Dose 2**	**Dose 3**	**Positive control**
Dose [mg/kg bw]		—	15	30	60	20
Number of evaluated polychromatic erythrocytes per animal		2000	2000	2000	2000	2000
Sampling time after last treatment (h)		24	24	24	24	24
Number of erythrocytes (mean of animals investigated)	normochromatic	2376	3889	4909	6926	2439
	polychromatic	2000	2000	2000	2000	2000
	polychromatic with micronuclei	3.2	2.2	6.8	3.6	21.8*
Ratio of erythrocytes	polychromatic / normochromatic	2000/2376	2000/3889	2000/4909	2000/6926	2000/2439

* $p < 0.01$ in non-parametric Wilcoxon ranking test

Table A6_6_4-1.B Table for micronucleus test in vivo (repeat test)

Two treatments at 24 h intervals		Negative control (vehicle)	Dose group
Dose [mg/kg bw]		—	2000
Number of evaluated polychromatic erythrocytes per animal		1000	1000
Sampling time after last treatment (h)		6	6
Number of erythrocytes (average of animals investigated)	normochromatic	1072.9	679.6
	polychromatic	1000	1000
	polychromatic with micronuclei	2.2	1.0
Ratio of erythrocytes	polychromatic / normochromatic	1000/1072.9	1000/679.6