Section A6.6.6 Genotoxicity in vivo

Annex Point IIA6.6 6.6.6 In-vivo determination of genotoxicity in germ cells of male mice (Rodent dominant lethal test)

Official use only

1 REFERENCE

1.1	**Reference**	[redacted], 1986, KUE 13032 C – Dichlofluanid - Dominant lethal test on the male mouse to assess for mutagenic effects, [redacted] Report No. [redacted] 1986-10-20 (unpublished)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 study used in this study are comparable with the OECD-Guideline 478 and carried out according to the recommendations of the ad hoc Chemogenetics Committee (Arch. Toxicol. 39, 173-185, 1978).
2.2	**GLP**	Yes
2.3	**Deviations**	Yes Deviations from the OECD-Guideline 478: - no positive control was 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]
3.1.2 3	Stability	The batch was analysed and approved for at least the duration of the study. The stability in test solvent gave no relevant indication of a change in the active ingredient.
3.1.2.4	Maximum tolerable dose	—
3.2	**Test Animals**	
3.2.1	Species	Mouse
3.2.2	Strain	Bor : NMRI (SPF Han)
3.2.3	Source	[redacted]
3.2.4	Sex	males + females

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Section A6.6.6 Genotoxicity in vivo

Annex Point IIA6.6 6.6.6 In-vivo determination of genotoxicity in germ cells of male mice (Rodent dominant lethal test)

3.2.5	Age/weight at study initiation	Males: Age: 8 – 12 weeks Weight: 33 - 45 g Females: Age: 8 – 12 weeks Weight: 25 - 30 g
3.2.6	Number of animals per group	50 males per group were treated. Approx. 600 females per male treatment group and control group were used for mating. Females were not treated.
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Number of applications	1
3.3.2	Interval between applications	—
3.3.3	Postexposure period	Mating: Starting with the day of administration, the bucks were mated at 12 intervals of 4 days each, a new untreated female being caged with each buck at the beginning of every interval. During these 48 days, all germ cell stages present in the testicles at the time of treatment could theoretically serve to inseminate and fertilize eggs. Examination of females: 14 days after the middle of the relevant mating interval, examinations were performed to determine pre- and post-implantation losses.
3.3.4	Type	
3.3.5	Concentration	0, 2500 or 5000 mg/kg bw
3.3.6	Vehicle	0.5 % aqueous Cremophor emulsion
3.3.7	Concentration in vehicle	0, 125 or 250 mg/ml
3.3.8	Total volume applied	20 ml/kg bw
3.3.9	Controls	Vehicle (negative control)

Section A6.6.6 Genotoxicity in vivo

Annex Point IIA6.6 6.6.6 In-vivo determination of genotoxicity in germ cells of male mice (Rodent dominant lethal test)

3.4	**Examinations**	
3.4.1	Clinical signs	Yes
3.4.2	Tissue	Uterine contents
	Number of animals:	all females
	Time points:	14 days after the middle of the relevant mating interval.
	Parameters:	Dead and live implants Total implants Corpora lutea Pre- and post-implantation losses Fertilisation rate
3.5	**Further remarks**	

4 RESULTS AND DISCUSSION

4.1	**Clinical signs**	Following acute oral administration of 2500 mg/kg or 5000 mg/kg bw dichlofluanid, the males displayed signs of illness, up to 72 hours apathy being observed in both groups. Narrowed or closed lids and feeble reflexes also occur. The animals behaved normally thereafter. Six males died in the 2500 mg/kg bw group and thirteen in the 5000 mg/kg bw group.
4.2	**Haematology / Tissue examination**	—
4.3	**Genotoxicity**	No

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Dichlofluanid was assessed for mutagenic effects in the dominant letal test following acute oral treatment of male mice. The methods used in this study were comparable with the OECD-Guideline 478. Existing deviations were described in 2.3. The administered dichlofluanid doses were 2500 mg/kg bw and 5000 mg/kg bw. The dose of the test substance based on a pilot study on male mice involving oral administration of 2000 mg/kg bw, 4000 mg/kg bw and 8000 mg/kg bw to groups of five animals, in which all doses led to symptoms. Those observed were reduced motility, rough fur, goose-stepping, gummy eyes, narrowed lids, larbored and in some cases slowed respiration. Two of the five treated animals died in the 4000 mg/kg bw group and three of five in the 8000 mg/kg bw group.

Section A6.6.6 Genotoxicity in vivo

Annex Point IIA6.6 6.6.6 In-vivo determination of genotoxicity in germ cells of male mice (Rodent dominant lethal test)

5.2	**Results and discussion**	Dichlofluanid did not lead to a permanent effect on the general behaviour of male mice following acute oral treatment with 2500 mg/kg bw and 5000 mg/kg bw in the dominant letal test. The treated mice displayed signs of illness for up to 72 hours, but their fertility was not affected. Substance related mortalities were observed in both dose groups. Statistical evaluation of the parameters significant for the assessment (dead and live implants, pre-implantation loss and total implants) showed no variation between the control group and the dose groups which would have had to be considered a negative effect of dichlofluanid.	
5.3	**Conclusion**	Assessment of dichlofluanid in the dominant lethal test on the male mice at acute oral doses of 2500 mg/kg bw and 5000 mg/kg bw provided no indications of mutagenic effects by the substance.	**X**
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	1/11/04
Materials and Methods	As described above [IUCLID 5.6 8/9]
Results and discussion	As described above
Conclusion	As no positive control was provided, it is uncertain whether the negative finding is a genuine result, or an indication that the animals were not responding.
Reliability	2
Acceptability	Acceptable
Remarks	The UK CA has added a comment regarding interpretation of the study, as a positive control substance was not included.
	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_4-2 Table for in vivo determination of genotoxicity in germ cells of male mice: Dominant lethal test

	Negative control	Low dose 2500 mg/kg bw	High dose 5000 mg/kg bw
Fertilisation rate* (%)	83.0	80.3	79.2
Results were the average of fertilised females of all mating intervals per dose group			
Total implants	12.5	12.6	12.5
Living implants	11.8	11.7	11.7
Dead implants	0.75	0.83	0.82
Corpora lutea	13.4	13.6	13.7
Pre-implantation loss	0.87	1.01	1 20

*the **fertilisation rate** is defined as the following percentage:

number of fertilised females per total number of females × 100