Section A6.8.1 Teratogenicity Study

Annex Point IIA6.8 6.8.1 Developmental toxicity test in the rat

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] 1989, A Teratology Study with Dichlofluanid (EUPAREN VM90), [redacted], 1989-01-04 (unpublished)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

2.1.1	Guideline study	Yes Methods used in this study are in accordance with the recommendations of the OECD-Guideline 414 and the EPA, Pesticide Assessment Guidelines, Subdivision F, series 83.3.
2.1.2	GLP	Yes
2.1.3	Deviations	Yes Compared with the OECD-Guideline 414, the following deviations could be ascertained: - A post-treatment period of 5 days before caesarean section→ only the period of organogenesis was examined under treatment conditions. - Food consumption was not recorded in three-day intervals during the treatment period, and the examination days did not coincide with the body weight examination days.

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	A stability study demonstrated that refrigerated 1.25 % and 5.0 % suspensions of the test article in the aqueous 0.5 % w/v Emulphor vehicle remained stable for at least 28 days with less than 5 % deviation from the initial concentration. This was a sufficient time interval to assure stability during entire exposure period. Each test suspension was homogenous and the concentration of dichlofluanid was within the established departmental limits of variation (± 10 %) for a test suspension formulation.
3.2	**Test Animals**	
3.2.1	Species	Rattus norvegicus

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3.2.2	Strain	Crl :CD BR
3.2.3	Source	[redacted]
3.2.4	Sex	Males (only for breeding) and females
3.2.5	Age/weight at study initiation	Males: Weight: 340 – 410 g Age: 12 weeks Females: Weight: 202 – 280 g Age: 11 weeks
3.2.6	Number of animals per group	28 inseminated females per group
3.2.7	Control animals	Yes
3.2.8	Mating period	Over night mating
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of exposure	Day 6-15 post mating
3.3.2	Post-exposure period	5 days (from day 16 until day 20 of gestation)
3.3.3	Type	Gavage
3.3.4	Concentration	Gavage 0, 125, 250 or 500 mg/kg bw
3.3.5	Vehicle	Aqueous solution (0.5% v/v Emulphor solution)
3.3.6	Concentration in vehicle	0, 12.5, 25.0, 50.0 mg/ml
3.3.7	Total volume applied	10.0 ml/kg bw
3.3.8	Controls	Vehicle
3.4	**Examinations**	
3.4.1	Body weight	Yes Days: 0, 6, 8, 10, 12, 15, and 20 of gestation.
3.4.2	Food consumption	Yes Days: 1, 6, 7, 12, 16, and 20 of gestation.
3.4.3	Clinical signs	Yes Daily.

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3.4.4	Examination of uterine content	Gravid uterine weight Number of corpora lutea Number of implantations Number of embryonic or foetal death and viable foetuses, and degree of resorption Organ weights: uterus, placenta Fertility index Gestation index Pre- and post-implantation loss
3.4.5	Examination of foetuses	
3.4.5.1	General	Litter Size, no. of dead foetuses, foetal weight, sex ratio, placenta weight, external alterations (overall body conformation, position of pinnae, size and position of eye bulges, examination of palate, examination of extremities),
3.4.5.2	Skelet	Yes
3.4.5.3	Soft tissue	Yes
3.5	**Further remarks**	—

4 RESULTS AND DISCUSSION

4.1	**Maternal toxic Effects**	For the mid- and high-dose groups, there was a statistically significant reduction in mean body weight gain between days 6 and 8 of gestation. At the high-dose level, there was an actual loss of body weight (final dam weight less the weight of the intact uterus) between days 6 and 8 of gestation. There was also a statistically significant reduction in mean actual body weight gain for the high-dose group when compared to the control group. Food consumption for the mid- and high-dose groups was significantly reduced on day 7 of gestation, when compared to the control group. After this initial reduction in food consumption, animals appeared to recover as with body weight and food consumption for both the mid- and the high-dose groups compared with the control group during the remainder of the study. No further effects occurred.
4.2	**Teratogenic / embryotoxic effects**	The test substance did not affect any maternal reproductive parameters, and did not cause embryotoxicity (increased resorption), foetotoxicity (decreased foetal weight or late gestational death), teratogenicity (increased malformations) at maternally toxic dose levels up to and including 500 mg/kg. Foetal sex ratios (expressed as the median percent of male foetuses) for each dose-level compared with the control group and were, except for a slightly low value of 43.2 for the high-dose group, which although below the historical control range (42.9 - 56.3) for this laboratory, was not considered to be meaningfully different from the control.
4.3	**Other effects**	—

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5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The study was conducted to determine the potential of dichlofluanid, administered orally by gavage, to induce maternal effects as well as to promote embryotoxicity, foetotoxicity, and/or teratogenicity in the rat. The methods in this study used were in accordance with the OECD-Guideline 414 and the recommendations contained in EPA, Pesticide Assessment Guidelines, Subdivision F, series 83.3. Slight deviations occurred and were described in 2.3 (see above).
5.2	**Results and discussion**	Dichlofluanid-related maternal toxicity was characterised by a reduction in body weight gain and food consumption at 250 and 500 mg/kg bw. The active substance did not affect maternal reproductive parameters and did not cause embryotoxicity (increased resorption), foetotoxicity (decreased foetal weight or late gestational death), teratogenicity (increased malformations) at any dose-level.
5.3	**Conclusion**	Dichlofluanid caused maternal toxicity at dose-levels of 250 and 500 mg/kg. However, the test substance did not affect any maternal reproductive parameters and appears to be devoid of any potential to promote embryotoxicity, foetotoxicity, and/or teratogenicity at maternally toxic dose-levels up to and including 500 mg/kg. A dose of 500 mg/kg is considered the no observed effect level (NOEL) for developmental toxicity. A dose of 125 mg/kg is considered the NOEL for maternal toxicity.
5.3.1	LO(A)EL maternal toxic effects	Reduced body weight gain and feed consumption; LOEL: 250 mg/kg bw.
5.3.2	NO(A)EL maternal toxic effects	NOEL: 125 mg/kg bw
5.3.3	LO(A)EL embryotoxic / teratogenic effects	—
5.3.4	NO(A)EL embryotoxic / teratogenic effects	NOEL: 500 mg/kg bw
5.3.5	Reliability	1
5.3.6	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	29/10/04
Materials and Methods	As described above [IUCLID 5.8.2 1/3]
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_8-1. Table for Teratogenic effects

Maternal effects

Parameter	Control data		Low dose	Medium dose	High dose	Dose-response
	Historical range	Study	125 mg/kg bw	250 mg/kg bw	500 mg/kg bw	+ / -
Number of dams examined		**27**	**27**	**26**	**26**	
Clinical findings during application of test substance		**No treatment-related clinical findings**				
Mortality of dams *(%)*	0%	**0%**	**0%**	**0%**	**0%**	**-**
Abortions		**0**	**0**	**0**	**0**	**-**
Mean body weight gain, *gain (mean ± s.e.)*						
day 6-8		**7.9 ± 0.9**	**5.6 ± 0.7**	**2.0* ± 1.1**	**-3.7*± 1.5**	**+**
day 8-10		**9.6 ± 1.0**	**10.1 ± 0.9**	**10.9 ± 1.2**	**13.4 ± 1.5**	**-**
day 6-15		**43.1 ± 2.2**	**43.9 ± 1.5**	**39.7 ± 2.2**	**37.2 ± 1.8**	**-**
day 0-end of test		**132.2 ± 4.2**	**133.1 ± 3.6**	**129.3 ± 3.6**	**121.3 ± 3.1**	**-**
final dam weight less weight of intact uterus		**54.0 ± 2.6**	**54.8 ± 2.4**	**51.3 ± 2.2**	**42.2**± 1.9**	**+**
Mean food consumption *(mean ± s.e.)*						
day 1		**20.7 ± 0.5**	**20.4 ± 0.6**	**20.4 ± 0.6**	**20.8 ± 0.8**	**-**
day 6		**22.2 ± 0.7**	**22.8 ± 0.5**	**22.5 ± 0.6**	**22.1 ± 0.8**	**-**
day 7		**21.0 ± 0.7**	**20.3 ± 0.7**	**15.7* ± 0.7**	**13.2* ± 0.7**	**+**
day 12		**24.8 ± 0.7**	**25.7 ± 0.6**	**23.6 ± 0.7**	**24.0 ± 0.6**	**-**
day 16		**26.0 ± 0.6**	**27.5 ± 0.6**	**27.0 ± 0.9**	**26.6 ± 0.6**	**-**
day 20		**26.5 ± 1.0**	**27.3 ± 0.7**	**28.3 ± 0.5**	**28.3 ± 0.5**	**-**
Pregnancies *pregnant animals/number of animals inseminated (% pregnancy)*	(68.0 –100)	**27/28 (96.4)**	**27/28 (96.4)**	**26/28 (92.9)**	**26/28 (92.9)**	**-**
Necropsy findings in dams dead before end of test		**No animals died before end of the test.**				

* difference against control $p \leq 0,05$ significant (Dunnett's test)

** difference against control $p \leq 0,01$significant (Dunnett's test)

Table A6_8-2. Table for Teratogenic effects

Litter response (Caesarean section data)

Parameter	Control data		Low dose 125 mg/kg bw	Medium dose 250 mg/kg bw	High dose 500 mg/kg bw	Dose-response + / -
	Historical range	Study				
Corpora lutea *mean (range)*	13.3-16.6 (1-26)	**15.3 (12-18)**	**16.3 (14-19)**	**15.8 (13-20)**	**15.6 (12-19)**	-
Implantations *total/number of dams*	mean 11.6-15.4	**397/27**	**417/27**	**390/26**	**384/26**	-
Resorptions *total/number of dams*	total 8-32	**24/27**	**45§/27**	**28/26**	**22/26**	-
Total number of foetuses	146-386	**373**	**372**	**362**	**362**	-
Pre-implantation loss mean *(%)*	3.0-18.8	**5.0**	**7.2**	**6.9**	**6.2**	-
Post-implantation loss *mean (%)*	2.8-13.7	**7.0**	**10.7**	**7.2**	**5.6**	-
Total number of litters	12-28	**27**	**27**	**26**	**26**	-
Foetuses / litter *mean (range)*	10.9-14.4 (1-20)	**13.8 (2-17)**	**13.8 (5-18)**	**13.9 (4-20)**	**13.9 (6-18)**	-
Total number of live foetuses		**373**	**372**	**361**	**362**	-
Total number of dead foetuses	0-8	**0**	**0**	**1**	**0**	-
Foetus weight (median) *[g]*	3.4-4.0	**3.6**	**3.7**	**3.6**	**3.6**	-
Placenta weight (median) *[g]*	0.5-0.55	**0.53**	**0.53**	**0.50***	**0.51**	-
Crown-rump length (mean) *[mm]*		**Not determined.**				
Median percent male foetuses	42.9-56.3	**50.0**	**53.3**	**53.6**	**42.3§**	-

* difference against control $p \leq 0,05$ significant (Kruskal-Wallis and Dunn's tests)

§ outside the historical control range

Table A6_8-3. Table for Teratogenic effects

Examination of the fetuses

Parameter	**Control data**		**Low dose 125 mg/kg bw**	**Medium dose 250 mg/kg bw**	**High dose 500 mg/kg bw**	**Dose-response + / -**
	Historical range	**Study**				
External malformations* *[%]*	0-11.5/ 0-0.9	**0/0**	**0/0**	**0/0**	**3.8/0.3**	**-**
Skeletal malformations* *[%]*	0-18.8/ 0-4.2	**0/0**	**0/0**	**3.8/0.5**	**11.5/1.6**	**-**
Skeletal variants* [%] extra ribs	10.7-66.7/ 0.5-15.7	**3.7/0.5**	**7.4/1.0**	**19.2/3.3**	**7.7/1.1**	**-**
additional pre-sacral vertebrae or sacral shift	0-66.7/ 0-1.9	**0/0**	**0/0**	**0/0**	**7.7/1.1**	**-**
Visceral malformations* *[%]*	0-24.0/ 0-3.5	**0/0**	**3.7/0.6**	**0/0**	**7.7/1.1**	**-**

***litter incidence/foetal incidence**