Section A6.8.1 Teratogenicity Study

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

Official use only

1 REFERENCE

1.1	**Reference**	[redacted], 1982, Embryotoxicity study in rabbits with oral application of KUE 13032 C (dichlofluanid, a.i. of Euparen), [redacted] Report No [redacted], 1982-11-05 (unpublished)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 Methods used in this study are comparable with the OECD-Guideline 414.
2.2	**GLP**	Yes
2.3	**Deviations**	Yes Compared with the OECD-Guideline 414 the following deviations could be ascertained: - Post-treatment period of 10 days before caesarean section→ only the period of organogenesis was examined under treatment conditions, - Test and control groups contained 13 – 15 dams with implantation sites instead of the recommended 16 animals with implantation sites, - Number of Corpora lutea and number and percent of pre- and post-implantation losses were not determined, - Historical control data 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	White powder
3.1.2.2	Purity	[redacted]
3.1.2.3	Stability	The chemical identity, purity, stability of the test substance as well as the homogeneity and concentration in the vehicle (0.5 % aqueous Cremophor EL) was determined and released.
3.2	**Test Animals**	
3.2.1	Species	Rabbit
3.2.2	Strain	Himalayan rabbit Chbb:HM
3.2.3	Source	[redacted]

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3.2.4	Sex	Males (only for breeding) and females
3.2.5	Age/weight at study initiation	Females: Weight: 2.0 – 2.6 kg Age: 18 -24 weeks
3.2.6	Number of animals per group	15 pregnant females per group
3.2.7	Control animals	Yes
3.2.8	Mating period	Mated naturally between 8 and 10 a m.
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of exposure	
		rabbit: day 6-18 post mating
3.3.2	Post-exposure period	10 days
3.3.3	Type	Gavage
3.3.4	Concentration	Gavage 0, 10, 30 or 100 mg/kg bw
3.3.5	Vehicle	0.5% aqueous Cremophor EL solution
3.3.6	Concentration in vehicle	0, 2.0, 6.0 or 20.0 mg/ml
3.3.7	Total volume applied	5 ml/kg bw
3.3.8	Controls	Vehicle
3.4	**Examinations**	
3.4.1	Body weight	Yes
3.4.2	Food consumption	Yes
3.4.3	Clinical signs	Yes
3.4.4	Examination of uterine content	Gravid uterine weight Number of implantations Number of embryonic or foetal death and viable foetuses and number of resorptions Mean placental weight of each litter and mean placental weight of each group
3.4.5	Examination of foetuses	
3.4.5.1	General	Litter Size, mean foetal weight of each litter and mean foetal weight of each group, incidence of runts (weight less than 26 g at section), sex ratio, external malformations, brain anomalies.
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

Appearance and behaviour of control and treated female rabbits were comparable to each other during gestation period, with the exception of one dam in the 100 mg/kg bw group that was wheezing on days 9, 10 and 11 of gestation.

Two dams of the 100 mg/kg bw group delivered prematurely. One dam delivered 7 live pups on day 23 p.c.; the other delivered 2 live pups on day 21 p.c.

Body weight:

During the treatment period (day 6 – 18) the mean body weight gain of dams receiving 10 and 30 dichlofluanid mg/kg bw was slightly but not statistically lower than that of the control group. In the 100 mg/kg dose group a significant mean body weight loss was observed.

In the post-treatment period (day 18 – 29) the mean body weight gain was, in comparison to the control group, slightly but not dose-dependently higher in the intermediate and high dose group.

During the entire gestation period (day 0- 29) dams of the intermediate dose group gained more, those of the low and high dose groups less than the control groups. The difference in comparison to the control group was statistically significant in the high dose group.

Food consumption:

During the treatment period (day 6-18) dams of the 100 mg/kg dose group consumed significantly less food than the control group.

During the post-treatment period (day 18-29) dams of all test groups consumed more food than the control group. The difference was significant only in the 30 mg/kg dose group.

During the entire gestation period there was no significant difference between the control and the test groups.

4.2 Teratogenic / embryotoxic effects

One dam in the 30 mg/kg dose group and one in the 100 mg/kg group had all their implantations resorbed.

The mean weight of the gravid uteri were lower in the dose groups, however the difference was statistically significant in the 10 mg/kg and 100 mg/kg dose groups. The mean placental weights were not affected.

The mean number of implantation sites and the mean number of live foetuses were lower in the dose groups; however, the difference was only statistically significant in the 100 mg/kg dose groups.

4.3 Other effects

—

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 rabbit.

The methods used in this study are comparable with the OECD-Guideline 414 with some deviations (see 2.3 above).

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5.2	**Results and discussion**	Pregnant rabbits tolerated oral administration of 10 and 30 mg/kg bw of dichlofluanid during organogenesis without evidence of maternal toxicity. Administration of 100 mg/kg induced moderate maternal toxicity as evidenced by a weight loss, reduced food intake and premature delivery of two litters. In the 100 mg/kg dose group the weight of the gravid uteri, the number of implantation sites and live foetuses were significantly reduced.
5.3	**Conclusion**	In conclusion, in reference to maternal, embryotoxic and/or foetotoxic effects, the doses of 10 and 30 mg/kg can be considered as no-effect doses; in reference to teratogenic effects all doses given are considered as no-effect doses in this study.
5.3.1	LO(A)EL maternal toxic effects	LOAEL: 100 mg/kg bw
5.3.2	NO(A)EL maternal toxic effects	NOAEL 30 mg/kg bw
5.3.3	LO(A)EL embryotoxic / teratogenic effects	LOAEL embryotoxic: 100 mg/kg bw
5.3.4	NO(A)EL embryotoxic / teratogenic effects	NOAEL embryotoxic 30 mg/kg bw NOAEL teratogenicity > 100 mg/kg bw
5.3.5	Reliability	2
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	9/08/06
Materials and Methods	As described above [IUCLID 5.8.2 3/3]
Results and discussion	During the dosing period high dose dams failed to gain weight (-52g, compared to +81g in controls). This effect on body weight gain remained evident at study termination (35 % decrease compared to controls). These effects on body weight gain were statistically significant.
Conclusion	The number of viable pups and implantation sites was significantly decreased at 100 mg/kg/day, highest dose used. It is not possible to determine whether the observed implantation loss represents a genuine effect, or incorrectly identified early resorption.
Reliability	2
Acceptability	Acceptable
Remarks	The UK CA agrees with the applicant's summary and conclusions. However, the UK CA has included information on body weight gain, as this suggests that general toxicity was the underlying cause of the implantation losses.
	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	Study	10 mg/kg bw	30 mg/kg bw	100 mg/kg bw	+ / -
Number of dams examined		**14**	**13**	**14**	**15**	
Clinical findings during application of test substance					**2 dams delivered prematurely 1 dam was wheezing on days 9-11 of gestation**	+
Mortality of dams		**0%**	**0%**	**0%**	**0%**	-
Abortions		**0**	**0**	**0**	**0**	-
Mean body weight gain *gain (mean ± S.D.)*						
day 6-18		**81± 59**	**59 ± 76**	**59 ± 107**	**-52** ± 129**	+
day 18-29		**193 ± 87**	**189 ± 63**	**256 ± 117**	**208 ± 104**	-
day 0-end of test		**328 ± 101**	**258 ± 85**	**351 ± 158**	**215* ± 124**	+
Mean food consumption [g] *(mean ± S.D.)*						
day 6-18		**1060 ± 246**	**1061 ± 245**	**1025 ± 331**	**671*** ± 228**	+
day 18-29		**1048 ± 161**	**1161 ± 167**	**1229** ± 151**	**1194 ± 219**	+
day 0-end of test		**2676 ± 357**	**2739 ± 318**	**2805 ± 476**	**2463 ± 404**	-
Pregnancies[#]		**14/15**	**13/15**	**14/15**	**15/15**	-
Necropsy findings in dams dead before end of test		**No animals died before study termination.**				

[#] pregnant animals / number of animals inseminated

* significantly different from controls, $p \leq 0.02$ (Student's t-test)

** significantly different from controls, $p \leq 0.01$ (Student's t-test)

*** significantly different from controls, $p \leq 0.001$ (Student's t-test)

Table A6_8-2. Table for Teratogenic effects

Litter response (Caesarean section data)

Parameter	Control data		Low dose	Medium dose	High dose	Dose-response
	Historical	Study	10 mg/kg bw	30 mg/kg bw	100 mg/kg bw	+ / -
Corpora lutea *total/number of dams*	**Not determined.**					
Implantations *mean ± S.D.*		**7.6 ± 1.7**	**6.3 ± 1.7**	**6.9 ± 3.0**	**5.4** ± 2.0**	+
Resorptions + dead foetuses *mean ± S.D.*		**0.6 ± 0.8**	**0.4 ± 0.6**	**0.6 ± 0.8**	**0.6 ± 0.8**	-
Total number of foetuses		**97**	**77**	**87**	**62**	+
Pre-implantation loss *state %*	**Not determined.**					
Post-implantation loss *state %*	**Not determined.**					
Number of litters		**14**	**13**	**14**	**13**	-
Foetuses / litter		**6.9 ± 1.6**	**5.9 ± 1.7**	**6.2 ± 3.2**	**4.8* ± 2.6**	+
Live foetuses / litter *(total/litter)*		**97/14**	**77/13**	**87/14**	**62/13**	-
Resorptions + dead foetuses / litter *(total/litter)*		**9/14**	**5/13**	**9/14**	**8/13**	-
Foetus weight *[g] (mean ± S.D.)*		**41.33 ± 5.25**	**40.21 ± 5.11**	**40.57 ± 3.88**	**40.31 ± 3.60**	-
Placenta weight *[g] (mean ± S.D.)*		**4.77 ± 0.49**	**4.87 ± 0.58**	**4.63 ± 0.49**	**4.93 ± 0.63**	-
Crown-rump length *[mm] (mean ± S.D.)*	**Not determined.**					
Fetal sex ratio *[ratio m/f](mean)*		**4.1/2.9**	**2.3/3.6**	**3.2/3.0**	**2.7/2.1**	-

* significantly different from controls, $p \leq 0.02$ (Student's t-test)

** significantly different from controls, $p \leq 0.01$ (Chi square test without Yates correction using a 2 x 2 contingency table)

Table A6_8-3. Table for Teratogenic effects

Examination of the fetuses

Parameter	Control data		Low dose	Medium dose	High dose	Dose-response
	Historical	**Study**	**10 mg/kg bw**	**30 mg/kg bw**	**100 mg/kg bw**	**+ / -**
Number of foetuses examined		**97**	**77**	**87**	**62**	
Skeletal anomalies[*1] *[%]*		**1.0**	**0**	**2.3**	**4.8**	**-**
Skeletal variants [%]						
5th sternebrae not ossified		**29**	**27**	**30**	**31**	-
13th rib point or comma shaped		**1**	**4**	**2**	**3**	-
Visceral anomalies[*2] *[%]*		**2.1**	**1.3**	**3.4**	**0**	**-**
Multiple malformations[*3] *[%]*		**0**	**0**	**1.1**	**0**	**-**

[*1] foetuses with asymmetric and/or asymmetric and fused sternebrae

[*2] foetuses cystic dilatations in diencephalon or with hydrocephalus internus

[*3] foetuses with dysmelia syndrome, abdominal eventration, spinabifida, cleft palate, skeletal muliple malformations