Section A6.8.1 (02) Teratogenicity Study

Annex Point IIA6.8.1. (02) **Teratogenicity test – rabbit, oral route**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]; WL 43467 (Cypermethrin) – Effects upon the progress and outcome of pregnancy in the rabbit; [redacted].	
		Dates of experimental work: 17 May 1983 – 28 July 1983 (preliminary study) and 11 August 1983 – 4 January 1984 (main study)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Chimac-Agriphar s.a.	
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 authorisation	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No guidelines mention in report, however protocol appears to be in compliance with method B.31 of Directive 87/302/EEC.	
2.2	**GLP**	No. GLP was not compulsory at the time the study was performed.	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	WL 43467 (Cypermethrin)	
3.1.1	Lot/Batch number	OCR/30 ST 76/001	
3.1.2	Specification	Deviating from specification given in section 2 as follows	
3.1.2.1	Description	Not mentioned in report	
3.1.2.2	Purity	97.5 % w/w	
3.1.2.3	Stability	Not mentioned in report	
3.2	**Test Animals**		
3.2.1	Species	Rabbit	
3.2.2	Strain	New Zealand White	
3.2.3	Source	[redacted]	
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	21-27 weeks minimum, 3.69 - 4.44 kg	
3.2.6	Number of animals per group	16 (control and 50 mg/kg dose group), 22 (20/mg/kg dose group), 17 (120 mg/kg dose group).	
		Additional animals were allocated to the 20 and 120 mg/kg dose groups to replaced dead animals or those removed from the study or not pregnant.	
3.2.7	Control animals	Yes. One vehicle control group (corn oil) and two positive control groups (thalidomide).	

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3.2.8	Mating period	Not applicable. Females were artificially inseminated and subsequently injected with 25 i.u. of lutenising hormone to ensure successful ovulation. The day of insemination was designated day 0 of gestation.
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of exposure	Days 6 to 18 of pregnancy
3.3.2	Postexposure period	11 days (animals killed on day 29 of gestation)
		Oral
3.3.3	Type	Gavage
3.3.4	Concentration	

Dose group (Cypermethrin mg/kg/d)	Volume-dosage of 20% w/v cypermethrin solution (ml/kg/d)
0	0.6
20	0.1
50	0.25
120	0.6

3.3.5	Vehicle	Corn oil
3.3.6	Concentration in vehicle	Test compound was prepared twice weekly as a 20% w/v cypermethrin solution in corn oil. Quantity to be supplied to the animals in each dose group was drawn from the relevant pre-formulated batch on a daily basis.
3.3.7	Total volume applied	Not reported. Dose administered daily was dependent on bodyweight of the individual animal on the day of dosing. All individual volumes were recorded.
3.3.8	Vehicle Control	Vehicle control animals received corn-oil only at a volume-dosage equivalent to that of the 120 mg/kg dose group (i.e. 0.6 ml/kg/d)
3.3.9	Positive Control	Two positive control groups were treated with Thalidomide, formulated freshly each day as a 0.5% w/v suspension in gum tragacanth mucilage. Animals were dosed daily by gavage (5 ml/kg) from days 6 to 18 of gestation.

Dose group (Thalidomide mg/kg/d)	No. of animals/group
125	16
150	10

3.4	**Examinations**	
3.4.1	Body weight	Yes
3.4.2	Food consumption	No
3.4.3	Clinical signs	Yes
3.4.4	Examination of uterine content	Number of corpora lutea

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		Number of implantations
		Number of resorption sites
		Number and distribution of live and dead foetus in each uterine horn
3.4.5	Examination of foetuses	
3.4.5.1	General	Litter Size, numberr. of dead foetuses, foetal weight and sex.
3.4.5.2	Skeletal	Yes
3.4.5.3	Soft tissue	Yes
3.5	**Further remarks**	Pre-implantation and Post-implantation % loss calculated
3.6	**Statistics**	Inter-group differences were to be assessed using appropriate statistical methods. In this particular study the only statistical test performed was on corrected day 29 maternal bodyweight (multiple t-test).

4 RESULTS AND DISCUSSION

4.1 Maternal toxic Effects

The general condition of control and treated females was comparable throughout the study. One animal from the control group, three from the 20 mg/kg group and two each from the 50 and 120 mg/kg groups either died or were killed in extremis due to respiratory tract infection and/or gastro-intestinal tract infection. None of the deaths were attributed to treatment with cypermethrin.

Some inter-group variations in maternal bodyweight gain were recorded but no adverse effects or treatment related trends were found. Corrected day 29 bodyweight showed a trend towards reduction with increased dosage, however this was not statistically significant.

Examination of all females at necropsy on day 29 of gestation revealed no macroscopic changes that could be related to treatment with cypermethrin. Litter responses were unaffected by treatment of the dams (see table A6_8_1_02.1).

All females successfully carried their young to term with the exception of two females in the 20mg/kg group and one in the 120 mg/kg which aborted during the post-treatment phase but none were found to be treatment related.

4.2 Teratogenic / embryotoxic effects

Examination of foetuses at necropsy, revealed a small number of abnormalities in all groups; the type and the incidences of which have previously been found to occur spontaneously in this strain of rabbit in other laboratory tests. (see Table A6_8_1_02-1)

4.3 Other effects

The number of implantations, live young and resorptions, pre- and post-implantation losses, foetal and placental weights were unaffected by treatment with cypermethrin at any dose level.

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5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Cypermethrin (batch number OCR/30 ST 76/001; purity 97.5%) was administered by oral gavage to pregnant New Zealand White rabbits from day 6 to day 18 of gestation inclusive at dose levels of 20, 50, and 120 mg/kg/day. A vehicle control group received corn oil at a volume dosage equal to that of the top dose group. Two positive control groups received thalidomide at 125 and 150 mg/kg/day. On day 29 of gestation the females were killed to allow examination of the uterine contents.
5.2	**Results and discussion**	The general condition of the treated females was comparable with that of the vehicle control throughout the study. Isolated deaths occurred in the control and each of the treatment groups but these were not treatment related. Some inter-group variations in bodyweight gain were recorded but no adverse effects or trends attributable to cypermethrin were found. Two females in the low dose group and one in the highest dose group aborted during the post exposure phase, however these were not treatment related. No macroscopic changes were observed in females at day 29 of gestation. Litter responses were unaffected. No foetal abnormalities were observed which could be attributed to treatment with the test substance.
5.3	**Conclusion**	There was no embryotoxicity or teratogenicity associated with oral administration of cypermethrin.
5.3.1	LO(A)EL maternal toxic effects	
5.3.2	NO(A)EL maternal toxic effects	120 mg/kg bw/d
5.3.3	LO(A)EL embryotoxic / teratogenic effects	
5.3.4	NO(A)EL embryotoxic / teratogenic effects	120 mg/kg bw/d
5.3.5	Reliability	2
5.3.6	Deficiencies	No. Study is not GLP, however it is considered robust and was carried out at a recognised facility and to an acceptable protocol using cypermethrin of known purity. Study has been evaluated and accepted under the Directive 91/414/EC.

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	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May, 2007.
Materials and Methods	The applicant's version is acceptable.
Results and discussion	The applicant's version is adopted with the following amendment: Table A6_8_1_02-1 is extended.
Conclusion	The applicant's version is adopted. There was no maternal toxicity, embryotoxicity or teratotoxicity associated with the oral administration of cypermethrin up to the dose of 120 mg/kg bw/d. NOAELmaternal = 120 mg/kg bw/d NOAELembryo/terato = 120 mg/kg bw/d
Reliability	2
Acceptability	Acceptable. Weakness of the study: MTD not reached. The dose levels have not been selected with the view to demonstrate any dosage related response. The highest dose did not induce observable maternal and/or developmental toxicity. However, the study is acceptable.
Remarks	
	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_02-1. Teratogenicity of cypermethrin in rabbit – Summary of maternal and foetal effects

Endpoint/dose (mg/kg bw/d)	**0**	**20 mg/kg bw**	**50 mg/kg bw**	**120 mg/kg bw**
Mortality	1	3	2	2
Number of animals inseminated	16	22	16	17
Number pregnant with live young day 29	14	10	13	12
body weight during dosing period	Trend towards reduction (not significant)			
Body weigt day 29 (kg)	4.40	4.38	4.37	4.36
Gravid uterus weight (g)	459	474	514	495
Premature sacrifice or deaths	1	3	2	2
Animals not pregnant	1	4	1	1
% abortion and total litter loss	0	16.7	0	7.7
Corpora lutea count	9.9	11.1	11.4	11.3
Implantations	8.4	8.3	9.8	8.9
Viable young M/F	4.1/3.1	3.7/4.2	4.5/3.8	4.1/4.3
Resorptions early/late	0.4/0.8	0.1/0.3	0.2/1.3	0.1/0.5
Implantation loss % : pre/post	15.8/13.7	25.2/4.8	13.5/14.8	20.7/6.5
Foetal weight (g)	43.2	41.5	41.8	42.4
Placenta weight (g)	6.5	5.5	5.9	5.8
Litter response	Unaffected by treatment			
Foetal observations External, soft tissue, skeletal alterations	No indication of adverse response			