Section A6.8.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA6.8.2 **3 Generation reproduction study – Rat**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]: Toxicity studies on the insecticide WL 43467(cypermethrin): A 3 generation reproduction study in rats; [redacted]	
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 available at the time this study was conducted. However the study appears comparable to method B.35 of Directive 87/302/EEC with some deviations.	
2.2	**GLP**	No GLP was not compulsory at the time the study was performed	
2.3	**Deviations**	Yes Methods used not fully in compliance with method B.35 of Directive 87/302/EEC. Males and females were exposed for 5 weeks : normally, in male rats, dosing is continued for 10 weeks prior to the mating period. Females must be dosed throughout the 3 week mating period, pregnancy and up to the weaning of the F1 offspring.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	WL 43467 (cypermethrin)	
3.1.1	Lot/Batch number	30	
3.1.2	Specification	Deviating from specification given in section 2 as follows	
3.1.2.1	Description	Not specified in report	
3.1.2.2	Purity	98%	
3.1.2.3	Stability	Not mentioned in report	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Wistar (SPF)	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male and Female	
3.2.5	Age/weight at study initiation	5 weeks, 47-50g	
3.2.6	Number of animals per group	30 males, 30 females	

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3.2.7	Mating	At 10 weeks of age, one female was randomly allocated to one male in the same treatments group (avoiding brother/sister pairings). Each pair were allowed to produce 2 litters (F1A and F1B). Litter F1A killed at weaning (21 days). One male and one female randomly selected from each of the weaned F1B litters and fed the appropriate diet. These rats were paired again at 10 weeks and allowed to produce 2 litters (F2A and F2B). A random selection of F2B offspring were mated to produce F3A and F3B litters.
3.2.8	Duration of mating	Each pair allowed to produce 2 offspring
3.2.9	Deviations from standard protocol	See section 2.3
3.2.10	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Animal assignment to dosage groups	30 male and 30 female animals in each treatment group
3.3.2	Duration of exposure before mating	5 weeks
3.3.3	Duration of exposure in general P, F1, F2 males, females	Dietary exposure to the test material was continuous for all generations from F0 prior to mating through to the weaning of the F2B generations.
3.3.4	Type	In food
3.3.5	Concentration	0, 10, 100 or 500 ppm Converted test article intake: 0, 1, 10, 50 mg/kg bw/d
3.3.6	Vehicle	Test article dissolved in acetone and gradually added to powdered LAD 2 diet.
3.3.7	Concentration in vehicle	For the 500 ppm dose level, 30g cypermethrin dissolved in in 60g acetone and gradually added to 60kg powdered LAD2 diet. The 100 and 10 ppm dose levels were prepared from 500ppm diet diluted with powdered LAD2.
3.3.8	Total volume applied	See above
3.3.9	Controls	Vehicle in LAD2 diet
3.4	**Examinations**	
3.4.1	Clinical signs	Yes
3.4.2	Body weight	Bodyweight measured weekly during pre-mating period and up to the age of 10 weeks. A further measurement was taken of male and females immediately before mating at F1 and F2.
3.4.3	Food/water consumption	Food intake measured weekly during pre-mating period and up to the age of 10 weeks.
3.4.4	Oestrus cycle	Ovaries examined microscopically
3.4.5	Sperm parameters	Testes examined microscopically

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3.4.6	Offspring	Date born Number of pups born alive Number of pups born dead Sex of pups alive on day 1 Number and sex of pre-weaned deaths Number and sex of pups weaned Total litter weights on days 1, 4, 7, 14 and 21 Individual pup bodyweights on day 21
3.4.7	Organ weights P and F1	Not mentioned in report
3.4.8	Histopathology P and F1	Not mentioned in report
3.4.9	Histopathology F1 not selected for mating, F2	Not mentioned in report
3.5	**Further remarks**	The majority of the microscopic findings in this study were confined to the adults of the F2B generation. Tissues examined were as follows: Brain (cerebrum, cerebellum, mid-brain, medulla), heart (ventricles), liver, spleen, kidneys, testes / ovaries, stomach, pancreas, lymph nodes, prostate / uterus, urinary bladder, thyroid, parathyroid, thymus, eye, lungs, pituitary, adrenals, small intestine (3 levels), large intestine (2 levels), oesophagus, salivary glands, sciatic nerve, any other tissues showing macroscopic lesions.
		4 RESULTS AND DISCUSSION
4.1	**Effects**	
4.1.1	**Adults**	F0, F1 and F2 body weights were lower at 500 ppm; at 10 or 100 ppm no significant effects were observed. The effects were greater for females than for males. Mean food intake of all F0 test groups were lower than controls and were probably related to unpalatability of the diet containing test material, week 3 being the first week of food intake . These lower food intakes at weeks 6 and 7 were associated with adverse body weight effects and were considered to be related to exposure to 500 ppm cypermethrin (Table A6_8_2-1). Fertility, gestation, viability, and lactation indices were similar for treated and control animals within each generation.

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4.1.2 Litters

At 500 ppm: Litter size and weights of F0A was reduced and these findings could be attributed to significantly lower F0A litter sizes recorded at this dose group. Mean pup weights were significantly lower compared with controls for F0B females pups and pups of both sexes and for F2B male pups.

At 100 and 10 ppm: the significantly lower litter sizes recorded at 10 ppm for F0A pups and F1B pups were not considered to be toxicologically relevant as similar changes were not observed at 100 ppm.

The significantly higher numbers of female pups per litter for the F0B litters were regarded as anomalous.

For the F0B pups the significantly higher mean litter weight, recorded for the 10 ppm groups at day 7, was related to a large mean litter size and appears to be anomalous.

For F2B litters , mean pup weights for male only were significantly lower at 100 and 10 ppm. This effect was regarded as anomalous. These results were re-analysed using the litter as experimental unit. This is considered to be statistically preferable approach for this type of analysis. Using the litter as experimental unit, no statistically significant differences were reported at 10 and 100 ppm. Within a given litter, the weight of an individual pup can be expected to be influenced by the number and weight of other pups in the litter as well as by any effect of the test compound. Therefore, these results were not taken into account for the NOAEL.

Significantly increased mean pup weights recorded at 10 ppm for the F0A litters, reflect the significantly lower litter sizes recorded at day 7, 14, 21. No significant differences in mean pup weights were recorded for the F1A, F1B or F2A litters at any dose levels.

(See Table A6_8_2-2)

4.2 Other

At the beginning of the study each treatment group comprised 30 pairs. However, as the study progressed the following factors caused a reduction of the treatment group sizes for certain litter varieties:

1) pairings not resulting in litters produced

2) single sex litters

3) pups of one sex or no pups surviving to weaning.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Wistar rats (SPF) received in the diet for 5 weeks, cypermethrin (98% B.n° : 30) at doses of 0, 10, 100, or 500ppm, each group comprised 34 females. Males and females from each treatment group were selected at random and caged together for mating. Two litters were produced from each pair for three successive generations. The second litter of each generation was weaned, fed the appropriate diet and mated to produce 2 litters.

Dietary exposure to the test material was continuous for all generations from F0 prior to mating through to the weaning of the F2B generations.

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5.2	**Results and discussion**	Reductions in bodyweight and food intake were seen at intervals in male and female rats in the 500ppm dose group for each generation. Pregnancy rates of treated and control groups were similar for each generation. No changes in litter size were seen in any generation in any dose group with the exception of a reduction in litter size in the 500ppm treated F0A litters on days 0, 7 and 21. No consistent changes in mean total litter weight with the exception of the 500ppm treated F0A litters on days 4, 14 and 21. Reduction in male and female pup weaning weights were seen in the 100 and 500ppm treatment groups from F0B. No compound-related gross or microscopical pathological findings were observed over three generations.	
5.3	**Conclusion**		
5.3.1	NO(A)EL		
5.3.1.1	Parents	100ppm = 10 mg/kg bw/d	
5.3.1.2	Reproduction	500ppm = 50 mg/kg bw/d	X
5.3.2	Reliability	2	
5.3.3	Deficiencies	Yes Methods used not fully in compliance with method B.35 of Directive 87/302/EEC. Males and females were exposed for 5 weeks, normally in male rats dosing is continued for 10 weeks prior to the mating period. Females are usually dosed throughout the 3 week mating period, pregnancy and up to the weaning of the F1 offspring. However, the study is considered scientifically sound and has been evaluated and accepted under 91/414/EC.	

	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: The effects observed in the pups are secondary to maternal toxicity. No effects were observed on fertility at any dose tested. NOAEL parental: 10 mg/kg bw/d NOAEL reproduction: 50 mg/kg bw/d NOAEL developmental: 10 mg/kg bw/d
Conclusion	The applicant's version is adopted with the following amendment: NOAEL parental: 10 mg/kg bw/d NOAEL reproduction: 50 mg/kg bw/d NOAEL developmental: 10 mg/kg bw/d.
Reliability	2
Acceptability	Acceptable.
Remarks	

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	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_2-1. Table for reproductive toxicity – adult data

Endpoint/dose	**0**		**10 ppm**		**100 ppm**		**500 ppm**	
Adult rat F0	♂	♀	♂	♀	♂	♀	♂	♀
Food intake			↘ wk3,4,5 (4%)		↘ wk3,4 (4%)		↘ wk3wk 7 (7%)	↘ wk3,4, 5,6,7 (6-7%)
Body weight							↘	↘ (4-5%)
Adult rat F1, F2								
Food intake			↘ wk3 (F1:8%)					↘ wk4→7(F1:6-8%) ↘ wk5,7(F2:11-16%)
Body weight							↘ (F1:4-5%)	↘ (F1:4-7%) (F2:5-6%)

Table A6_8_2-2. Table Table for reproductive toxicity – litter data

Endpoint/dose	**0 ppm**	**10 ppm**	**100 ppm**	**500 ppm**
Litter survival:				↘ F0A day 0, 7,21
Litter size:				
F0A				↘ day, 7➔21
FOB, F1A, F1B, F2A,F2B		no effect		
Number ♀ pups/litter				
F0A				↘ day 1& 21
F0B		↗ day 1&21	↗ day 1&21	
F1A,F1B,F2A,F2B		no effect		
Mean litter weights:				
F0A				↘ day 4, 14,21
F0B		↗ day 7		
F1A, F1B,F2A,F2B		no effect		
Mean pup weight:				
FOA		↗ day 14&21		
F0B				_: ↘ 10% day 21
F1A, F1B, F2A		no effect		
F2B		↘ 10% day 21♂	↘ 11% day 21♂	↘ day 21♂ : 9% ♀: 6%

Analysis of covariance followed by Williams or Dunnett's test () : not biologically significant