Section A6.5, A6.7 Chronic Toxicity / Carcinogenicity

Annex Point IIA6.5, IIA6.7 **Rat-combined chronic toxicity/carcinogenicity study**

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted]; Toxicity studies on the insecticide WL 43467 (cypermethrin): A 2 year feeding 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 Existing study. Method used comparable to method B.30 of Directive 87/302/EEC (corresponding OECD guideline 453)
2.2	**GLP**	No GLP was not compulsory at the time the study was performed
2.3	**Deviations**	Yes Low number of rats. Blood albumin, glucose, GGT and ornithine decarboxylase were not measured. Urinalysis was not performed.
		3 MATERIALS AND METHODS
3.1	**Test material**	WL 43467 (cypermethrin), cis:trans ratio:1/1
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	Liquid
3.1.2.2	Purity	98%
3.1.2.3	Stability	Not specified in report
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Wistar
3.2.3	Source	[redacted]
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	5 weeks
3.2.6	Number of animals per group	24 animals/sex/dose

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3.2.6.1	at interim sacrifice	16 animals/sex/dose sacrificed at 6 or 12 months
		12 animals/sex/dose sacrificed at 18 months
3.2.6.2	at terminal sacrifice	24 animals/sex/dose sacrificed after 2 year exposure period
3.2.7	Control animals	Yes (48 animals/sex)
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	24 months
3.3.2	Interim sacrifice(s)	After 6, 12 and 18 months
3.3.3	Final sacrifice	After 24 months
3.3.4	Freqency of exposure	Daily
3.3.5	Postexposure period	None
		Oral
3.3.6	Type	In food
3.3.7	Concentration	0, 1, 10, 100, 1000 ppm (0, 0.05, 0.5, 5, 50 mg/kg bw) food consumption ad libitum
3.3.8	Vehicle	For 1000ppm diet: dissolved in a minimal amount (100ml) of A.R. grade acetone
		For 100, 10, 1 ppm diets: further dilution of 1000ppm diet with LAD 2. diet
3.3.9	Concentration in vehicle	See above
3.3.10	Total volume applied	See above
3.3.11	Controls	Vehicle plus LAD 2 diet
3.4	**Examinations**	
3.4.1	Body weight	Yes (weekly for the first 13 weeks, thereafter at four weekly intervals)
3.4.2	Food consumption	Yes (weekly for the first 13 weeks, thereafter at four weekly intervals)
3.4.3	Water consumption	No
3.4.4	Clinical signs	Yes
3.4.5	Macroscopic investigations	Tumour incidence and classification
3.4.6	Ophthalmoscopic examination	No
3.4.7	Haematology	Yes

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		Number of animals:	all animals
		Time points:	End of study/necropsy (6, 12 18 and 24 months)
		Parameters:	Total red and white cell counts, haemoglobin concentration and mean cell volumes.
			Differential white cell counts performed on top dose, and control group plus other dose levels if haematological abnormalities observed. Also on the 100ppm group at the 6 moth necropsy.
			Citrated blood used to estimate prothrombin and kaolin-cephalin coagulation times.
3.4.8	Clinical Chemistry	Yes	
		Number of animals:	All animals
		Time points:	End of study/necropsy (6, 12 18 and 24 months)
		Parameters:	Total plasma protein, urea and chloride levels, alkaline phosphatase activity, AST/ALT activity, plasma sodium/potassium levels.
3.4.9	Urinalysis	No	
3.4.10	Pathology	Yes	
3.4.10.1	Organ Weights	Yes	
		From:	all surviving animals
		Organs:	Liver, kidneys, testes, spleen, brain, heart
3.4.11	Histopathology	Yes	
		from:	All animals in the control and two highest dose groups (including those dying early)
			All animals in the lower dose groups in the 2 year group
		Organs:	Brain, pituitary, thyroid, parathyroid, thymus, oesophagus, salivary glands, stomach, small and large intestines, liver, pancreas, kidneys, adrenals, spleen, heart, trachea, lungs, gonads, prostate or uterus, urinary bladder, mesenteric lymph nodes, sciatic nerve, eye and lachrimal glands
			Tissues stored for reference: Knee joint and femur, femoral muscle, mammary gland, seminal vesicles, spinal cord, tongue, bone marrow smear.
3.4.12	Other examinations	None	

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3.5	**Statistics**	Body and organ weights were analysed by covariance analysis using initial bodyweight or terminal organ weight as the covariate respectively. Reported means were adjusted for covariance, where no significant covariance relationship was found unadjusted means were reported. Clinical chemical and haematological parameters were examined using analysis of variance. Any significant differences between the treated and control group means was tested using the Williams t-test. On occasions where a monotonic dose response could not be assumed, the Dunnett's test was used.
3.6	**Further remarks**	-
		4 RESULTS AND DISCUSSION
4.1	**Body weight**	Reduction in bodyweight seen in top dose 2 year males and females. For top dose animals in the interim groups, the reduction was statistically significant from weeks 1 to 32 (males), and 1 to 46 and week 76 (females)
4.2	**Food consumption**	Small reductions (not always statistically significant) in food consumption were seen during the first 13 weeks in the 1000 ppm group (both 2 year and ad interim animals).
4.3	**Water consumption**	Not determined
4.4	**Clinical signs**	No effects considered to be of toxicological significance
4.5	**Macroscopic investigations**	No effects considered to be of toxicological significance
4.6	**Ophthalmoscopic examination**	Not determined
4.7	**Haematology**	Minor fluctuations were seen in various parameters in the interim and 2 year groups but these were not considered to be of toxicological significance as they were minor in nature with no evidence of compound related tissue damage.
4.8	**Clinical Chemistry**	No effects considered to be of toxicological significance
4.9	**Urinalysis**	Not determined
4.10	**Pathology**	No compound related gross pathological changes in any tissues
4.11	**Organ Weights**	See table A6 5-1
4.12	**Histopathology**	Sciatic nerves from many animals at 1 year and later showed very small numbers of nerve fibres exhibiting the changes of Wallerian degeneration. The incidences increased with age but there was no difference in severity between the dose groups.
4.13	**Other examinations**	None
4.14	**Time to tumours**	See table A6 5-2. Statistical analysis showed no evidence of increased risk of tumour development over the 2 year period.

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4.15	**Other**	The most common tumour was pituitary adenoma, which occurred in 39 of 48 female controls in the 2 year group and was the cause of early death or removal from the trial in 19 of these. Similar incidences were found in other dose levels in females. These were present, but much less common, in males and were believed not to be treatment-related. A cluster of 4 uterine tumours in the 100 ppm was reported. Since there were no uterine tumours at 1000 ppm and there was no question of competing toxicity at the higher level preventing their development. These uterine tumours at 100 ppm were not considered to be compound related. Statistical analysis revealed no evidence of increased risk of tumour development following dietary inclusion of cypermethrin for up to 2 years.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	24 Wistar rats/sex/dose received in the diet WL43467(cypermethrin) (B.n°.30, 98%; cis/trans ratio : 1/1) at 0, 1, 10, 100, 1000 ppm for approximately 24 months. 48 animals/sex were used in the control group. Additional groups of 6 rats/sex/dose were sacrificed after 6 or 12 months and 12 rats/sex/dose were sacrificed after 18 months. Only 24 rats/sex/dose for the 2 year exposure period. Mean cypermethrin content of diets fed to rats over a 2 year period: 1.04, 10.0, 99.0 and 1002 ppm. Converted dose: : 0, 0.05, 0.5, 5 and 50 mg/kg/d .
5.2	**Results and discussion**	Minor fluctuations were seen in various haematological parameters in the interim and 2 year groups but these were not considered to be of toxicological significance.
5.3	**Conclusion**	Cypermethrin is not carcinogenic in this study NOAEL = 100 ppm = 5 mg/kg bw/day
5.3.1	Reliability	2
5.3.2	Deficiencies	Yes. Low number of rats ; blood albumin, glucose, GGT and ornithine decarboxylase were not measured. Urinalysis was not performed. However the study is considered acceptable as it was conducted at an established facility and used cypermethrin of known purity. Study evaluated and accepted under 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	April, 2007.
Materials and Methods	The applicant's version is acceptable.
Results and discussion	The applicant's version is adopted.
Conclusion	NOAEL = 100 ppm = 5 mg/kg bw/d The applicant's version is adopted.
Reliability	2
Acceptability	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_5-1. Table for 2 year toxicity study of cypermethrin in rats

Endpoint/dose	**0**		**1 ppm**		**10 ppm**		**100 ppm**		**1000 ppm**	
Survival rate (%)	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
6 months	100	100	100	100	100	100	100	100	100	100
12 months	100	83	100	100	100	83	100	100	100	100
18 months	100	71	92	83	75	67	100	83	100	100
24 months	67	42	46	33	54	38	71	42	71	50
Clinical signs	sore hocks , gross external masses, mammary tumors, general poor conditions and loss of appetite, not compound related									
Food consumption									(↘ 7%)	(↘ 10%)
Body weight									↘ 7%	↘ 7% w 1-75
Hematology: PT										↗ 4%
Clinical chemistry:										
liver PNOD									↗	↗
urea									↗ 58% 2 y	
AP					↘ 16% 2 y		↘ 18% 2 y		↘ 33% 2 y	
Na^+									↗ 1%	
Rel. organ weight										
testes									abs↘ 6 mth	
liver									abs+rel ↗ 18 mth	
heart									rel↗ 6 mth, abs↘ 12 mth	
kidney							abs↗ 18 mth		rel↗ 12mth, abs↗ 18 mth	rel↗ 6 mth
Histopathology: sciatic nerve degeneration : number affected/number survivors										
at 12 mth	2/12	1/12					0/6	2/6	1/6	2/6
at 18 mth	9/24	2/17					4/12	2/10	5/12	0/12
at 24 mth	17/31	10/20	8/11	3/8	11/13	4/9	10/17	5/9	12/17	5/12
Total	28/67	13/49					14/35	9/24	18/35	7/30

%	42	26.5					40	37.5	51.4	23

Statistically significant ↗ or↘ : covariance analysis; variance analysis; Williams t test or Dunnett's test

() not statistically significant

Table A6_5-2 Table for tumour incidence

Tissue/pathological finding % affected animals	**0**		**1 ppm**		**10 ppm**		**100 ppm**		**1000 ppm**	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Pituitary										
anterior lobe adenoma (TA) (B)	33	79	20	91	29	100	20	79	25	75
anterior lobe carcinoma (M)		6.2	4.1		4.1		4.1		4.1	
intermediate lobe adenoma (B)					4.1					
posterior lobe-asrrocytoma (M)	2.08								4.1	
Mammary glands										
fibroadenoma (B)		14				33		16		
adenocarcinoma (M)		28		63		33		16		40
Uterus										
adenocarcinoma (M)				4.1				4.1		
endometrial sarcoma (M)								8		

(B) benign; (M) malignant