Section A6.4.1 (01) Repeated dose toxicity

Annex Point IIA 6.4.1

Subchronic Oral Toxicity - Rat

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted];Toxicity studies on the insecticide WL 43467 (cypermethrin): three month feeding study in rats; [redacted].	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Chimac-Agriphar s.a.	
1.2.2			
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 partly in compliance with method B.26 of Directive 87/302/EEC and OECD guideline 408 (1981)	
2.2	**GLP**	No GLP was not compulsory at the time the study was performed	
2.3	**Deviations**	Yes Histopathology not performed on all organs. Target organs not examined at all dose levels.	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	WL 43467 (cypermethrin)	
3.1.1	Lot/Batch number	21	
3.1.2	Specification	Deviating from specification given in section 2 as follows	
3.1.2.1	Description	liquid	
3.1.2.2	Purity	98.5%	
3.1.2.3	Stability	Stable	X
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	CD (SPF)	
3.2.3	Source	[redacted]	
3.2.4	Sex	Males and females	
3.2.5	Age/weight at study initiation	5 weeks	
3.2.6	Number of animals per group	12 female rats per treatment group (11 for 25 ppm group) 24 males and 23 females in each control group respectively	X
3.2.7	Control animals	Yes	

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3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	90 days
3.3.2	Frequency of exposure	Daily
3.3.3	Postexposure period	None
3.3.4	**Oral**	
3.3.4.1	Type	In food
3.3.4.2	Concentration	0, 1.25, 5, 20, and 80 mg/kg /d ad libitum (0, 25, 100, 400 and 1600 ppm)
3.3.4.3	Vehicle	Not mentioned in report
3.3.4.4	Concentration in vehicle	Not mentioned in report
3.3.4.5	Total volume applied	Not mentioned in report
3.3.4.6	Controls	vehicle, plain diet or other
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes (daily observation)
3.4.1.2	Mortality	Yes (recorded daily)
3.4.2	Body weight	Yes (recorded weekly)
3.4.3	Food consumption	Yes (recorded weekly)
3.4.4	Water consumption	No
3.4.5	Ophthalmoscopic examination	No
3.4.6	Haematology	Yes (at necropsy)
3.4.7	Clinical Chemisty	Yes (at necropsy)
3.4.8	Urinalysis	No
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	Yes organs: liver, kidneys, testes, spleen, brain, heart
3.5.2	Gross and histopathology	Yes Control, 400 and 1600 ppm groups organs: brain, heart, kidney, lung, spleen, liver, alimentary tract, pancreas, salivary gland, thymus, mesenteric lymph node, gonads, prostate or uterus, pituatry, adrenals, larynx, thyroid, eye and sciatic nerve.

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3.5.3	Other examinations	None
3.5.4	Statistics	Yes, Body and organ weights analysed by covariance. Chemical and haematological parameters examined using analysis of variance.
3.6	**Further remarks**	-
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	In the 1600 ppm group, many of the animals showed hypersensitivity and abnormal gait during the first 5 weeks of the experiment. Clinical recovery was observed after the end of the 5th week and their food intake increased to a normal level. The general health and behaviour of animals in dose groups up to 400 ppm were unaffected by ingestion of cypermethrin.
4.1.2	Mortality	In the 1600 ppm group , 1 male died and 3 were killed for humane reasons.
4.2	**Body weight gain**	See table A6.4.1_01_1
4.3	**Food consumption and compound intake**	See table A6.4.1_01_1
4.4	**Ophtalmoscopic examination**	Not performed
4.5	**Blood analysis**	
4.5.1	Haematology	See table A6.4.1_01_1
4.5.2	Clinical chemistry	See table A6.4.1_01_1
4.5.3	Urinalysis	Not performed
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	See table A6.4.1_01_1
4.6.2	Gross and histopathology	Two of the male rats in the 1600 ppm group showed axon breaks and vacuolation of myelin in the sciatic nerve.
4.7	**Other**	The MTD was reached
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	12 CD (SPF) rats/sex were fed a diet containing cypermethrin, WL 43467 (B.n°.21, 98.5 %) at concentrations of 0, 25, 100, 400 and 1600 ppm over a period of 13 weeks (converted dose: 1.25, 5, 20 and 80 mg/kg/d).

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5.2	**Results and discussion**	The MTD was reached. In the 1600 ppm group many of the animals showed hypersensitivity and abnormal gait during the first 5 weeks of the experiment. Of these group, 1 male died and 3 were killed for humane reasons. Two of these rats showed axon breaks and vacuolation of myelin in the sciatic nerve. Clinical recovery was observed after the end of the 5th week and their food intake increased to a normal level. The general health and behaviour of animals in dose groups up to 400 ppm were unaffected by ingestion of cypermethrin. .
5.3	**Conclusion**	
5.3.1	LO(A)EL	
5.3.2	NO(A)EL	100 ppm = 5 mg/kg bw, later updated to 20 mg/kg bw (see point 5.3.5 below)
5.3.3	Other	
5.3.4	Reliability	2
5.3.5	Deficiencies	Yes. Protocol not fully in compliance with test method B of Directive 87/302/EEC or OECD guideline 408 (1981). However the study is considered acceptable as it was carried out at an established facility and using cypermethrin of known purity. Study evaluated and accepted under Directive 91/414/EC. The NOAEL of 5 mg/kg was assigned in the original monograph. However during the ECCO review process this was subsequently changed to 20 mg/kg bw.

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 accepted with the following amendments: *2.3 Deviations:* No examination of water consumption, urinalysis, ophtalmoscopic examination. *3.1.2.3 Stability:* The stability of the test substance in the diet was not confirmed. *3.2.6 Number of animals per group:* 12 male and female rats per treatment group (except 11 females for the 25ppm group)
Results and discussion	The applicant's version is accepted.
Conclusion	The applicant's version is accepted: NO(A)EL: 20 mg/kg bw (400 ppm)
Reliability	2
Acceptability	acceptable

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Remarks	
	COMMENTS FROM ... *(specify)*
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_4_1_01_1: Rat-90 day feeding study – Results

Endpoinst/dose	**0**		**25 ppm**		**100 ppm**		**400 ppm**		**1600 ppm**	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Mortality									3	
Clinical signs									ataxia, spayed hind limbs hypersensitivity	
Body weight									↘ 17%	↘ 10%
Food intake									↘	↘
Hematology										
Hb									↘ 4%	↘ 6%
PCV										↘ 7%
RBC										↘ 6%
KCCT									↘ 11%	
Clinical chemistry										
Proteins										↘ 6%
K+									↗ 13%	
AP										↗ 40%
Urea									↗ 20%	↗ 39%
Mean Organ weight (adjusted for terminal bodyweight)										
Liver										↗ 10%
Kidney							↗ 5%		↗ 7%	↗ 14%
Spleen										↗ 17%