Section A6.6.4 Genotoxicity in vivo

Annex Point IIA 6.6.4 **In-vivo bone marrow micronucleus study in mice**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]; Mouse bone marrow micronucleus test of test substance Cypermethrin cis:trans/40:60; [redacted] Dates of experimental work: 8 December 1998 – 10 December 1998	
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**	Yes. OECD guideline no. 474 (1987)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Cypermethrin cis:trans/40:60	
3.1.1	Lot/Batch number	1503084	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description	Pale brown viscous liquid / semi-solid	
3.1.2.2	Purity	94.4% w/w	
3.1.2.3	Stability	Stable	
3.1.2.4	Maximum tolerable dose	100 mg/kg, determined in a range finding study.	
3.2	**Test Animals**		
3.2.1	Species	Mouse	
3.2.2	Strain	CRL:NMRI BR	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	8 weeks 25.0-29.7g (males) 22.0-27.7g (females)	
3.2.6	Number of animals per group	5 males + 5 females per dose and sampling time	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral (test article) Intraperitoneal (positive control)	
3.3.1	Number of applications	1	

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3.3.2	Interval between applications	Not applicable
3.3.3	Postexposure period	24 and 48 h after treatment (pos. control: 48 h after treatment; untreated control: 24 h after beginning of study)
		Oral
3.3.4	Type	Stomach tube (test substance) or gavage (vehicle control)
3.3.5	Concentration	100 mg/kg (MTD), 75 mg/kg (75% MTD) and 50 mg/kg (50% MTD)
3.3.6	Vehicle	Sunflower oil
3.3.7	Concentration in vehicle	1.0, 0.75 and 0.5% w/v
3.3.8	Total volume applied	0.1 ml/10g bw
3.3.9	Controls	Untreated control Sunflower oil (vehicle control) Cyclophosphamide 60 mg/kg (positive control)
3.4	**Examinations**	
3.4.1	Clinical signs	No
3.4.2	Tissue	bone marrow Number of animals: all animals Number of cells: 2000 Time points: 24 or 48 h after treatment Type of cells erythrocytes in bone marrow Parameters: Micronucleated PCEs polychromatic/normochromatic erythrocytes ratio
		4 RESULTS AND DISCUSSION
4.1	**Clinical signs**	Not determined
4.2	**Haematology / Tissue examination**	Test substance did not induce significant increase in the number of micronucleated PCEs in either males or females at any dose level 24 hours after treatment. After 48 hours, mathematically but not biologically significant increases in the number of MPCEs were seen in male mice at the 50 and 100 mg/kg dose and in females at the 75 mg/kg dose level. No differences in the ratio of polychromatic and normochromatic erythrocytes were found after treatment and significant depression of the PCE:NCE ratio was not observed. See table A6_6_4-1
4.3	**Genotoxicity**	Cypermethrin cis:trans/40:60 proved to be negative for mutagenicity in NMRI mice
4.4	**Other**	In the positive control, cyclophosphamide caused significant increase in the number of MPCEs 48 hours after application, thus validating the

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		test.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Potential mutagenicity of Cypermethrin cis:trans/40:60 was examined in bone marrow of NMRI mice according to OECD guideline 474. Test substance was applied at three dose levels; 100, 75 and 50 mg/kg. Following a range-finding study, the MTD was found to be 100 mg/kg. In the main study, animals were treated once via the oral route and samples were taken 24 and 48 hours after treatment. During the microscopic evaluation, 2000 PCEs were scored per animal to assess the micronucleated cells.	
5.2	**Results and discussion**	Single doses of 100, 75 and 50 mg/kg did not induce an increase in the frequency of micronucleated polychromatic erythrocytes (MCPEs) in male and female mice at 24 and 48 hours after treatment when compared to the vehicle control. No difference in the ratio of polychromatic to normochromatic erythrocytes occurred when compared to the vehicle control.	X
5.3	**Conclusion**	Cypermethrin cis:trans/40:60 proved to be negative for mutagenicity in the mouse in-vivo bone marrow micronucleus test.	
5.3.1	Reliability	1	
5.3.2	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	April, 2007.
Materials and Methods	The applicant's version is acceptable with the following amendment: 3.1.2.3 Stability: The stability data of the test substance in the vehicle was not reported.
Results and discussion	Revised version: Table A6_6_4-1 is adapted. Signs of toxicity are not reported for the main study. Single doses of 100, 75 and 50 mg/kg did not induce an increase in the frequency of micronucleated polychromatic erythrocytes (MCPEs) in male and female mice at 24 hours after treatment when compared to the vehicle control. However 48 hours after treatment, statistically significant increases in the number of MPCEs were seen in male mice at the 50 and 100 mg/kg dose and in females at the 75 mg/kg dose level. Nevertheless, the biological relevance of the seen increases is questionable.
Conclusion	The applicant's version is adopted. Under the test conditions in this study, cypermethrin cis:trans/40:60 does not produce micronuclei in the immature erythrocytes of NMRI mice, and as such proved to be negative for mutagenicity.

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Reliability	1
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_6_4-1. **Table for Mouse Micronucleus Test In Vivo**

Sex: Male

	Untreated control	**Positive control**	**Vehicle control**		**low dose 50 mg/kg**		**mid dose 75 mg/kg**		**high dose 100 mg/kg**	
No. of PCEs evaluated	**10000**	**10000**	**10000**		**10000**		**10000**		**10000**	
Sampling time (h)	**24**	**48**	**24**	**48**	**24**	**48**	**24**	**48**	**24**	**48**
PCE/NCE rate	1.27	0.31	1.18	1.18	1.12	1.08	1.14	1.11	1.08	1.07
MPCE (mean ±SD)	2.80 ± 0.45	32.60** ± 5.73	3.80 ± 0.45	3.40 ± 0.55	3.40 ± 1.14	4.40* ± 0.55	4.00 ± 1.00	3.60 ± 0.55	4.80 ± 0.84	4.60* ± 0.89

Sex: Female

	Untreated control	**Positive control**	**Vehicle control**		**low dose 50 mg/kg**		**mid dose 75 mg/kg**		**high dose 100 mg/kg**	
No. of PCEs evaluated	**10000**	**10000**	**10000**		**10000**		**10000**		**10000**	
Sampling time (h)	**24**	**48**	**24**	**48**	**24**	**48**	**24**	**48**	**24**	**48**
PCE/NCE rate	1.24	0.34	1.21	1.25	1.13	1.19	1.18	1.11	1.11	1.07
MPCE (mean ±SD)	2.80 ± 0.84	32.80** ± 3.56	3.40 ± 0.89	3.60 ± 0.55	3.40 ± 0.55	3.80 ± 0.45	4.20 ± 0.84	4.60* ± 0.55	4.40 ± 0.55	4.8 ± 1.30

* $p<0.05$

** $p<0.01$ (Kruskal-Wallis Non Parametric Anova)