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]; Genotoxicity evaluation of Cypermethrin technical by *in vivo* mouse micronucleus assay. [redacted] Dates of experimental work: 9 October 2008 – 15 November 2008	
1.2	**Data protection**	Yes	
1.2.1	Data owner	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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes. OECD guideline no. 474 (1997)	
2.2	**GLP**	No information included in study report to indicate GLP compliance.	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Cypermethrin technical	
3.1.1	Lot/Batch number	CMN92T1193AR	
3.1.2	Specification	Certificate of Analysis supplied with study report verified the batch was within accepted specification.	
3.1.2.1	Description	Viscous yellowish liquid	
3.1.2.2	Purity	93.5%	
3.1.2.3	Stability	Stable – expiry date 19 June 2010	
3.1.2.4	Maximum tolerable dose	50 mg/kg, determined in a range finding study.	
3.2	**Test Animals**		
3.2.1	Species	Mouse	
3.2.2	Strain	Swiss albino	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	6-8 weeks 23-27g (males and females)	
3.2.6	Number of animals per group	5 males and 5 females per dose and sampling time. A total of 55 males and 55 females allocated to 11 treatment or control groups.	
3.2.7	Control animals	Yes – vehicle/negative controls and positive control group	
3.3	**Administration/ Exposure**	Oral in dose volume not exceeding 2 mL/100g bodyweight. Vehicle was corn oil	
3.3.1	Number of applications	1 (one high dose group and the corresponding negative control received two doses on consecutive days)	

Section A6.6.4 Genotoxicity in vivo

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

3.3.2	Interval between applications	Not applicable except for one high dose group and the corresponding negative control that received two doses on consecutive days with 24 h interval.
3.3.3	Post-exposure period	24 and 48 h after treatment Positive control: 24 h after treatment; untreated control: 24 and 48 h after beginning of study and 24 hours after second treatment of one group. Low, intermediate and high dose groups – 24 and 48 h after initiation of treatment and for one high dose group, 24 h after second administration.
		Oral
3.3.4	Type	Gavage
3.3.5	Concentration	50 mg/kg (MTD), 25 mg/kg and 12.5 mg/kg
3.3.6	Vehicle	Corn oil
3.3.7	Concentration in vehicle	2.5, 1.25 and 0.625% w/v
3.3.8	Total volume applied	2 ml/10g bw
3.3.9	Controls	Corn oil (vehicle control) Cyclophosphamide 20 mg/kg (positive control)
3.4	**Examinations**	
3.4.1	Clinical signs	General health condition recorded daily, bodyweights recorded pre-treatment on Day 0.
3.4.2	Tissue	bone marrow
		Number of animals: all animals
		Number of cells: 2000 PCE examined. The PCE:NCE ratio determined from 200 erythrocytes
		Time points: 24 or 48 h after treatment
		Type of cells erythrocytes in bone marrow (PCE – immature or NCE - mature)
		Parameters: Micronucleated PCEs polychromatic/normochromatic erythrocytes ratio
		4 RESULTS AND DISCUSSION
4.1	**Clinical signs**	No signs of toxicity are presented in the study report, even at the MTD. Some indications of observed toxicity are given for the range-finding investigation but these are not replicated in the main study.

Section A6.6.4 Genotoxicity in vivo

Annex Point IIA 6.6.4

In-vivo bone marrow micronucleus study in mice

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 or 48 hours after single treatment, or 24 hours after two administrations. The mean percentage of micronucleated polychromatic erythrocytes in the treated groups was consistently lower than the vehicle mean for males and females at the 24 h and 48 h assessment points in the low and intermediate groups and the high dose groups were similar to the controls.

The ratio of PCE:NCE is theoretically anticipated to be close to unity, with higher ratios normally apparent in younger animals and a shift in the ratio indicates inhibition of cell division, a reduction in the PCE count and normally a reduction of the PCE:NCE ratio to less than 1.

The PCE:NCE ratios for the negative controls were:
24 h males and females: 1.35 and 1.42
48 h males and females: 1.37 and 1.43
24 h after two treatments: 1.47 and 1.45

The PCE:NCE ratios for the positive controls were:
24 h males and females: 1.75 and 1.83

The PCE:NCE ratios for the low and intermediate dose treated group were:
24 or 48 h males and females: in range of 0.86 to 1.28 (indicating a slight reduction in the ration in comparison with controls)

The PCE:NCE ratios for the high dose treated group were:
24 or 48 h males and females: in range of 0.69 to 0.85 after one or two administrations (indicating a significant reduction in the PCE:NCE ratio).

See table A6_6_4-1

There were no marked differences in the frequencies of micronucleated polychromatic erythrocytes for either sex in any of the treatment groups.

4.3 Genotoxicity

Cypermethrin technical proved to be negative for mutagenicity in Swiss albino mice

4.4 Other

In the positive control group, cyclophosphamide caused a significant increase in the number of micronucleated PCEs 24 hours after application, thus validating the test.

Section A6.6.4 Genotoxicity in vivo

Annex Point IIA 6.6.4

In-vivo bone marrow micronucleus study in mice

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Potential mutagenicity of Cypermethrin technical was examined in bone marrow of Swiss albino mice according to OECD test guideline 474. The *in vivo* micronucleus assay assesses the clastogenic and aneugenic potential of the test substance, which was administered orally at three dose levels; 50, 25 and 12.5 mg/kg. Following a range-finding study, the MTD was found to be 50 mg/kg. In the main study, animals were treated once via the oral route (corn oil used as the vehicle) and samples were taken 24 and 48 hours after treatment. One group of vehicle controls and a high dose group were treated on two occasions and samples collected 24 hours after the second dosing occasion. Cyclophosphamide was included as a standard positive control for the assay. During the microscopic evaluation, 2000 PCEs were scored per animal to assess the micronucleated cells. The PCE:NCE ratio was evaluated from assessment of 200 erythrocytes per group.	
5.2	**Results and discussion**	Single oral doses of 50, 25 or 12.5 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. The positive control induced a statistically significant increase in MPCE thereby validating the techniques used in the assay. The ratio of polychromatic to normochromatic erythrocytes was similar to the vehicle controls for the low and intermediate treatment regimen, although a slight reduction was evident for the treated groups at both sampling time points. The high dose group showed a reduction in the ratio also with a significantly lower ratio at 50 mg/kg compared to the vehicle control.	X
5.3	**Conclusion**	Cypermethrin technical induced no apparent chromosomal or other change leading to formation of micronuclei in polychromatic erythrocytes at dose levels up to the MTD of 50 mg/kg. Cypermethrin technical was not considered to be genotoxic and 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

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	January, 2011.
Materials and Methods	The applicant’s version is acceptable.
Results and discussion	The applicant’s version is adopted.
Conclusion	The applicant’s version is adopted.
Reliability	1
Acceptability	acceptable
Remarks	

Section A6.6.4 Genotoxicity in vivo

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

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

Males	**Positive control**		**Vehicle control**		**low dose 12.5 mg/kg**		**mid dose 25 mg/kg**		**high dose 50 mg/kg**	
Sampling time (h)	**24**	**48**	**24**	**48**	**24**	**48**	**24**	**48**	**24**	**48**
Total PCE scored	497	na	573	577	521	526	477	499	415	429
Total NCE scored	503	na	427	423	479	474	523	501	585	571
PCE/NCE ratio	1.01	na	1.35	1.37	1.11	1.12	0.92	1.02	0.72	0.76
Total MPCE scored	175	na	54	45	40	42	42	40	58	43
%MPCE (mean ±SD)	1.75 ± 0.47	na	0.54 ± 0.16	0.45 ± 0.14	0.40 ± 0.19	0.42 ± 0.22	0.42 ± 0.19	0.40 ± 0.20	0.58 ± 0.13	0.43 ± 0.16

Females	**Positive control**		**Vehicle control**		**low dose 12.5 mg/kg**		**mid dose 25 mg/kg**		**high dose 50 mg/kg**	
Sampling time (h)	**24**	**48**	**24**	**48**	**24**	**48**	**24**	**48**	**24**	**48**
Total PCE scored	520	na	587	587	511	560	489	452	408	452
Total NCE scored	480	na	413	413	489	440	511	548	592	548
PCE/NCE ratio	1.10	na	1.42	1.43	1.06	1.28	0.98	0.86	0.69	0.85
Total MPCE scored	183	na	46	33	49	39	42	34	55	42
MPCE (mean ±SD)	1.83 ± 0.61	na	0.46 ± 0.17	0.33 ± 0.18	0.49 ± 0.10	0.39 ± 0.11	0.42 ± 0.21	0.34 ± 0.15	0.55 ± 0.29	0.42 ± 0.15

Two day treatment – sampled 24 hours after dosing completed

Males and females	**Vehicle control**		**High dose 50 mg/kg**	
Sex	**M**	**F**	**M**	**F**
Total PCE scored	594	589	435	416
Total NCE scored	406	411	565	584
PCE/NCE ratio	1.47	1.45	0.77	0.72
Total MPCE scored	32	35	40	46
%MPCE (mean ±SD)	0.32± 0.16	0.35± 0.17	0.40± 0.20	0.46± 0.12