Section A6.6.2 Genotoxicity in vitro

Annex Point IIA 6.6.2 **In-vitro cytogenetic study in mammalian cells**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]; In vitro mammalian chromosomal aberration study of Cypermethrin cis:trans/40:60; [redacted] (unpublished) Dates of experimental work: 10 July 2001 – 25 July 2001	
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 473 (1997)	
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	2001044557	
3.1.2	Specification	Cis:trans = 40.2/59.8	
3.1.2.1	Description	Pale yellow viscous liquid	
3.1.2.2	Purity	93.6% w/w	
3.1.2.3	Stability	Stability not given	
3.2	**Study Type**	In Vitro mammalian chromosome aberration test	
3.2.1	Organism/cell type	Chinese hamster Ovary (CHO), subline KI	
3.2.2	Deficiencies / Proficiencies	Not applicable	
3.2.3	Metabolic activation system	S9 mix Male Wistar rats were treated orally by gavage with Phenobarbitone (PB) and β-naphtoflavone (BNF) (80 mg/kg respectively) on days 3,2 and 1 before sacrifice on day 5. Livers were removed aseptically, washed in 0.15M KCl and homogenised. Homogenates were centrifuged and the supernatant decanted and retained as the S9 fraction.	
3.2.4	Positive control	Ethyl methanesulphonate and N-Nitrosodimethylamine	
3.3	**Administration / Exposure; Application of test substance**		
3.3.1	Concentrations	10, 50 and 100 µg/ml Doses were selected on the basis of a preliminary cytotoxicity assay using concentrations of 1, 10, 50, 100, 200, 400, 800 and 1600 µg/ml cypermethrin in the presence and absence of S9 mix.	

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3.3.2	Way of application	The study was conducted as two separate experiments Study 1 The test substance cypermethrin was dissolved in dimethylsulphoxide (DMSO) (up to 10 mg/ml). Duplicate cultures were used for each test concentration and the vehicle control. $2x10^6$ seeded cells (10^6 cells/dish) were treated in each group. The exposure period was 4 hours at 37ºC, after which the cells were washed twice with F12 medium. The growth medium was then added. Samples were taken at approximately 1.5 normal cell cycle from the beginning of treatment. Study 2 As above, however the exposure period was 1.5 normal cell cycle length. After the exposure period, cells were washed twice with F12 medium and then sampled.	
3.3.3	Pre-incubation time	The Exposure period was 4 hours at 37ºC (study 1) or 1.5 normal cell cycle length = 18-20 hours (study 2). Cell cultures were treated with 0.5 µg/ml colchicines for 2 hours prior to harvesting. Cells were then swollen with 0.075M KCl and washed three times in fixative (3: 1/methanol:acetic acid). Once the preparation was free of plasma, cells were placed on slides and air dried.	
3.4	**Examinations**		
3.4.1	Number of cells evaluated	Slide preparations were Giemsa stained and all slides were scored blind. 200 metaphase cells containing 2N±2 centromeres were evaluated for structural aberrations in each experimental group. Chromatid and chromosome aberrations were recorded separately (including gaps, deletions and exchanges).	
3.5	**Statistics**	CHI^2 test was used for statistical analysis.	
		4 RESULTS AND DISCUSSION	
4.1	**Genotoxicity**	See tables A6_6_2-1 and A6_6_2-2	X
4.1.1	without metabolic activation	No significant increase in the number of aberrations without gaps	
4.1.2	with metabolic activation	No significant increase in the number of aberrations without gaps	
4.2	**Controls**	Both positive controls (ethyl methanesulphonate and N-nitrosodimethylamine) showed a clear clastogenic effect, validating the test. In the untreated control, the number of aberrations without gaps was <5%, demonstrating suitability of the cell line used in the study.	

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5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Test item was studied for clastogenic activity in CHO cells both in the presence and absence of metabolic activation. Two independent experiments were performed with an exposure period of 4 hours and 1.5 normal cell cycle length respectively (18-20 hours).
5.2	**Results and discussion**	There was no significant increase in the number of aberrations without gaps in either the presence or absence of metabolic activation in either study. The positive controls showed a clear clastogenic effect thereby validating the test.
5.3	**Conclusion**	Cypermethrin cis:trans/40:60 proved to be non-clastogenic
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.
Results and discussion	The applicant's version is adopted with the following amendments: The tables A6_6_2-1 and A6_6_2-2 are adapted and table A6_6_2-3 is added.
Conclusion	Cypermethrin cis:trans/40:60 does not induce chromosome aberrations, under the test conditions used, in cultured CHO-cells.
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_2-1. Chromosome Aberration Assay: Experiment 1, exposure time = 4 hours at 37ºC

		Untreated control		Vehicle control		Pos Control*		low dose 10µg/ml		mid dose 50µg/m		high dose 100µg/m	
		a	b	a	b	a	b	a	b	a	b	a	b
Without S9													
chromosome aberrations	gap	1	1	1	2	7	4	2	2	1	4	5	2
	del	0	0	0	0	4	7	1	0	0	0	1	1
	exchange	1	1	0	1	2	1	0	0	1	0	0	0
chromatid aberrations	gap	2	3	6	3	17	22	5	7	8	4	5	4
	del	1	1	2	1	14	16	1	1	1	1	1	0
	exchange	0	0	0	0	1	11	0	0	0	0	1	1
No. cells with aberrations (gap+)		3	4	6	5	18	17	7	8	8	7	9	6
No. cells with aberrations (gap-)		2	2	2	2	5	7	2	1	2	1	2	1
Abberation rate (aberrations/100 cells)		0.02	0.02	0.02	0.02	0.21	0.35	0.02	0.01	0.02	0.01	0.03	0.02
With S9													
chromosome aberrations	gap	2	3	1	2	6	3	2	4	1	4	2	2
	del	0	0	1	0	8	5	0	0	1	0	0	0
	exchange	0	0	0	1	3	2	1	0	0	0	1	1
chromatid aberrations	gap	2	2	3	4	19	14	6	3	8	3	3	6
	del	1	1	1	1	17	14	0	1	0	2	3	1
	exchange	0	0	0	0	2	0	0	0	0	0	0	0
No. cells with aberrations (gap+)		4	5	4	6	17	14	7	7	7	6	5	8
No. cells with aberrations (gap-)		1	1	1	1	10	12	1	1	1	1	3	2
Abberation rate (aberrations/100 cells)		0.01	0.01	0.02	0.02	0.30	0.21	0.01	0.01	0.01	0.02	0.04	0.02

* N-Nitrosodimethylamine (0.4µl/ml)

Table A6_6_2-2. Chromosome Aberration Assay: Experiment 2, exposure time = 18-20 hours at 37ºC

		Untreated control		Vehicle control		low dose 10µg/ml		mid dose 50µg/m		high dose 100µg/m	
		a	b	a	b	a	b	a	b	a	b
Without S9											
chromosome aberrations	gap	0	2	3	1	1	0	2	3	1	2
	del	0	0	0	0	1	1	0	0	1	0
	exchange	0	0	1	1	1	0	2	1	2	1
chromatid aberrations	gap	3	2	5	7	5	5	6	6	4	4
	del	1	1	1	1	2	1	0	0	1	2
	exchange	0	0	0	0	0	0	0	0	0	0
No. cells with aberrations (gap+)		4	4	6	8	5	5	8	9	5	6
No. cells with aberrations (gap-)		1	1	1	1	3	1	2	0	3	2
Abberation rate (aberrations/100 cells)		0.01	0.01	0.02	0.02	0.04	0.02	0.02	0.01	0.04	0.03
With S9											
chromosome aberrations	gap	1	2	1	1	2	1	2	2	1	2
	del	0	0	0	0	0	0	0	0	0	0
	exchange	0	0	0	0	1	0	1	0	0	0
chromatid aberrations	gap	4	2	6	5	2	4	3	3	8	4
	del	1	0	2	2	1	2	2	2	1	3
	exchange	0	0	0	0	0	0	0	2	1	0
No. cells with aberrations (gap+)		5	4	6	6	4	5	5	5	8	6
No. cells with aberrations (gap-)		1	04	2	2	1	2	3	3	1	2
Abberation rate (aberrations/100 cells)		0.01	0.00	0.02	0.02	0.02	0.02	0.03	0.04	0.02	0.03

Table A6_6_2-3. CHO Clonal toxicity data (treatment: 4h; cells seeded per dish: 200)					
Experiment 1					
Test condition	Dish 1	Dish 2	Dish 3	Average count ± SD	Relative survival (percent)*
Untreated control	204	206	209	206.33 ±2.52	10.81
Vehicle control (DMSO)	205	202	207	204.67 ± 2.52	100.00
Cypermethrin					
1 µg/ml	205	194	202	200.33 ± 5.69	97.88
10 µg/ml	181	192	198	190.33 ± 8.62	93.00
50 µg/ml	170	178	164	170.67 ± 7.02	83.39
100 µg/ml	92	103	98	97.67 ± 5.51	47.72
200 µg/ml	51	43	41	45 ± 5.29	21.99
400 µg/ml	0	0	0	0.00 ± 0.00	0.00
800 µg/ml	0	0	0	0.00 ± 0.00	0.00
1600 µg/ml	0	0	0	0.00 ± 0.00	0.00
Experiment 2					
Test condition	Dish 1	Dish 2	Dish 3	Average count ± SD	Relative survival (percent)
Untreated control	210	203	196	203.00 ± 7.00	100.83
Vehicle control (DMSO)	198	204	202	201.33 ± 3.06	100.00
Cypermethrin					
1 µg/ml	203	192	190	195.00 ± 7.00	96.41
10 µg/ml	180	173	181	178.00 ±4.36	88.41
50 µg/ml	162	161	170	164.33 ± 4.93	81.62
100 µg/ml	94	102	89	95.00 ± 6.56	47.19
200 µg/ml	19	21	22	20.67 ± 1.53	10.26
400 µg/ml	0	0	0	0.00 ± 0.00	0.00
800 µg/ml	0	0	0	0.00 ± 0.00	0.00
1600 µg/ml	0	0	0	0.00 ± 0.00	0.00

* Relative to vehicle control