Section A6.4.3 **Subchronic toxicity**
Annex Point IIA6.4 **90-day inhalation toxicity study in the rat**

not available as recommended in the guideline.

However, this deviation is not considered to compromise the scientific validity of the study.

23.2 Results and discussion

The mean actual chamber concentration to which groups of animals were exposed, were 0.1149 (range 0.0428 – 0.1541), 0.2201 (range 0.1367 – 0.2947), 0.4363 (range 0.2967 – 0.5500) and 0.4363 (range 0.2967 – 0.5500) mg Permethrin/L air, respectively. The concentrations were considered to be consistent during the study.

During the exposure period, 75.86 to 99.08% of the particles generated were < 5.8 µm at 0.1149 mg/L. 48.98 to 97.89% of the particles generated were < 5.8 µm at 0.2201 mg/L. 43.39 to 82.17% of the particles generated were < 5.8 µm at 0.4363 mg/L. Please refer to Table A6.4.3-1.

No mortality was reported in any group throughout the study.

No signs of toxicity were observed at 0, 0.1149 and 0.2201 mg/L. However, animals displayed toxicity signs such as nasal irritation and mild tremor at 0.4363 mg/L. These signs were noted on day 7 and disappeared on day 14 and 21 in the male and female animals respectively.

No treatment related significant changes in body weights were reported at 0, 0.1149 and 0.2201 mg/L. Decreases in body weights at 0.4363 and in the high dose satellite control group were significant at week 2. However body weight gain in males was similar to control animals from week 3 until study termination. Similarly, body weight gain in females was similar to controls from week 5 until termination. Results are summarised in Tables A6.4.3-2 and A6.4.3-3.

No changes in food consumption were recorded throughout the study.

No treatment related haematological and clinical chemistry changes were noted in any treated groups when compared to control.

At gross and histopathological examination, animals did not display any treatment related lesions.

Statistically significant changes in liver, kidneys and adrenals weights were reported in both sexes. However, they were not considered to be adverse and were not deemed to be of toxicological significance. Results are summarised in Tables A6.4.3-4, A6.4.3-5, A6.4.3-6 and A6.4.3-7.

23.3 Conclusion

Based upon toxicity signs such as nasal iritation and mild tremor at 0.4363 mg/L, the subchronic inhalation NOEL of Permetrhin technical for Wistar rats can be considered as 0.2201 mg/L.

Section A6.4.3	Subchronic toxicity	
Annex Point IIA6.4	90-day inhalation toxicity study in the rat	
23.3.1	LO(A)EL	0.4363 mg/L
23.3.2	NOEL	0.2201 mg/L
23.3.3	Reliability	1
23.3.4	Deficiencies	One deviation was noted and outlined under points 2.3 and 5.1. However it does not compromise the scientific validity of the study.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	*26th June 2009*
Materials and Methods	Accept the applicants version with following comments 2.3 The study was performed using technical rather than high purity material. 2.3 The study summary states that haematological and clinical chemistry data was not available. However, this data is given in the study report. 3.2.5 The weight of 90g for rats starting the study appears low and may suggest juvenile rather than mature animals
Results and discussion	Adopt applicant version.
Conclusion	LO(A)EL: 0.4363 mg/L NO(A)EL: 0.2201 mg/L *Based upon toxicity signs such as nasal iritation and mild tremor at 0.4363 mg/L.* Other conclusions: None Adopt applicant's version or include revised version
Reliability	1
Acceptability	Acceptable
Remarks	Statistically significant changes in liver, adrenal, gonad and kidney weights (absolute and relative) are seen in the low and mid dose groups. Changes are not seen in the control or high dose group and there appears to be no dose relationship. Consequently, it is difficult to determine the toxicological significance of these changes. There is no evidence of effect for either clinical chemistry results or hostopathology results. It is possible the weight effects are an artefact of the gross necropsy process? The only effect seen in clinical chemistry is decrease in BUN.
	Comments from ... *(SPECIFY)*
Date	*Give date of comments submitted*

Section A6.4.3 Subchronic toxicity

Annex Point IIA6.4 **90-day inhalation toxicity study in the rat**

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 A6.4.3-1: Mean exposure levels and particle size distribution measurements

Exposure concentration (mg/L)	Range of exposure concentration	Particles < 5.8 µm
0.1149	0.0428 – 0.1541	75.86 – 99.08%
0.2201	0.1367 – 0.2947	48.98 – 97.89%
0.4363	0.2967 – 0.5500	43.39 – 82.17%

Table A6.4.3-2: Summary of body weight values in grams (males)

Week of study	Air control	0.1149 mg/L	0.2201 mg/L	0.4363 mg/L	Satellite control	Satellite 0.4363 mg/L
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
0	100.90 ± 3.87	102.40 ± 3.66	102.20 ± 4.21	102.90 ± 4.63	102.90 ± 4.38	105.00 ± 3.50
1	114.70 ± 6.60	117.50 ± 5.52	118.40 ± 6.42	115.50 ± 6.47	114.20 ± 7.24	118.20 ± 6.41
2	123.40 ± 11.50	121.20 ±5.53	131.40 ± 10.51	121.00 ± 11.23[a]	127.60 ± 13.73	111.0 ± 15.31[a]
3	135.90 ± 13.88	122.90 ±12.42	131.80 ± 15.02	121.10 ± 11.25	138.10 ± 16.20	129.70 ± 16.89
4	137.70 ± 13.06	125.90 ±14.25	129.10 ± 9.80	120.20 ± 13.11	134.67 ± 7.71	129.40 ± 14.38
5	145.70 ± 13.34	145.00 ±13.49	135.10 ± 15.77	141.20 ± 27.61	154.90 ± 14.45	142.80 ± 14.20
6	156.90 ± 21.44	155.20 ± 20.31	148.30 ± 22.04	144.20 ± 16.70	167.30 ± 16.26	146.80 ± 17.04
7	170.40 ± 23.86	165.20 ± 20.11	154.60 ± 20.93	156.40 ± 17.11	180.40 ± 20.93	153.90 ± 19.14
8	182.20 ± 14.32	184.40 ± 18.88	173.40 ± 15.28	187.90 ± 14.69	184.50 ± 21.00	187.70 ± 26.00
9	203.90 ± 15.07	205.60 ± 16.69	187.40 ± 15.91	203.00 ± 12.32	191.90 ± 20.99	211.50 ± 31.58
10	220.50 ± 12.24	218.50 ± 17.15	200.80 ± 15.54	201.00 ± 14.19	206.10 ± 19.08	213.20 ± 26.45
11	236.30 ± 9.80	223.90 ± 19.01	212.30 ± 16.55	215.10 ± 19.44	215.90 ± 21.24	228.00 ± 29.73
12	244.80 ± 12.37	231.60 ± 18.74	224.40 ± 18.02	222.40 ± 18.39	231.70 ± 18.06	224.90 ± 34.36
13	262.40 ± 12.18	248.40 ± 21.18	239.30 ± 9.07	242.10 ± 13.18	250.50 ± 18.86	240.50 ± 32.70
14	-	-	-	-	261.40 ± 18.39	253.60 ± 28.13
15	-	-	-	-	263.40 ± 21.38	261.90 ± 27.07
16	-	-	-	-	274.90 ± 20.32	267.30 ± 34.24
17	-	-	-	-	292.00 ± 18.76	280.90 ± 33.02

values are expressed as mean ± SD (n=10)
[a] statistically different ($p<0.05$)

Table A6.4.3-3: Summary of body weight values in grams (females)

Week of study	Air control	0.1149 mg/L	0.2201 mg/L	0.4363 mg/L	Satellite control	Satellite 0.4363 mg/L
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
0	101.40 ± 3.66	102.60 ± 4.60	102.00 ± 3.46	101.00 ± 2.11	102.20 ± 3.68	103.30 ± 4.00
1	114.50 ± 4.95	116.00 ± 5.98	115.30 ± 7.21	113.90 ± 5.76	116.40 ± 5.64	116.70 ± 5.25
2	119.20 ± 9.86	120.90 ± 4.79	108.20 ± 7.32	86.20 ± 10.11[a]	112.70 ± 16.58	94.90 ± 5.95[a]
3	121.80 ± 8.56	127.30 ± 4.11	127.40 ± 6.93	95.50 ± 9.91[a]	118.20 ± 16.65	103.00 ± 5.52[a]
4	128.40 ± 5.13	124.40 ± 5.97	124.90 ± 6.64	112.60 ± 11.44[a]	120.30 ± 17.30	114.40 ± 5.62[a]
5	133.40 ± 4.67	133.20 ± 6.23	135.80 ± 10.01	120.60 ± 21.46	131.50 ± 17.20	134.90 ± 7.46
6	138.60 ± 6.11	136.30 ± 7.54	137.60 ± 14.15	131.40 ± 7.12	133.20 ± 16.52	139.00 ± 10.17
7	143.40 ± 9.73	146.00 ± 11.81	145.30 ± 16.17	142.60 ± 8.64	138.90 ± 17.18	145.00 ± 12.01
8	153.50 ± 8.14	153.80 ± 9.24	147.50 ± 25.21	146.50 ± 7.46	156.80 ± 12.30	147.20 ± 12.23
9	153.90 ± 7.32	162.70 ± 8.67	161.60 ± 21.53	162.00 ± 13.37	166.30 ± 14.45	161.80 ± 14.61
10	180.70 ± 11.12	179.70 ± 15.65	166.00 ± 16.89	178.20 ± 15.88	179.50 ± 15.09	169.50 ± 11.88
11	189.80 ± 8.40	188.40 ± 11.37	181.10 ± 15.12	192.90 ± 12.78	192.80 ± 14.32	194.10 ± 8.24
12	206.50 ± 13.02	193.90 ± 14.68	192.10 ± 18.73	210.20 ± 12.57	205.00 ± 13.32	191.30 ± 12.89
13	230.70 ± 15.29	210.20 ± 15.48	211.50 ± 14.88	221.10 ± 14.65	221.90 ± 13.80	213.30 ± 21.51
14	-	-	-	-	232.10 ± 12.11	221.70 ± 15.77
15	-	-	-	-	247.00 ± 11.16	236.30 ± 13.08
16	-	-	-	-	260.30 ± 13.51	255.70 ± 15.48
17	-	-	-	-	275.40 ± 16.31	268.70 ± 19.01

values are expressed as mean ± SD (n=10)
[a] Statistically different from control ($p<0.05$)

Table A6.4.3-4: Summary of absolute organ weight (g) – Male rats

Group/ Dose	Liver	Adrenal left	Adrenal right	Kidney left	Kidney right	Gonad left	Gonad right	Lungs
0 mg/L	8.163 ± 1.152	0.029 ± 0.004	0.028 ± 0.003	1.039 ± 0.135	1.036 ± 0.096	1.380 ± 0.068	1.361± 0.060	2.060 ± 0.096
0.1149 mg/L	9.039 ± 1.101	0.028 ± 0.004	0.030 ± 0.002	1.075 ± 0.137	1.074 ± 0.138	1.355 ± 0.128	1.367 ± 0.090	2.008 ± 0.173
0.2201 mg/L	7.931 ± 1.205	0.032 ± 0.003	0.031 ± 0.002	1.064 ± 0.122	1.112 ± 0.153	1.395 ± 0.117	1.334 ± 0.123	2.014 ± 0.161
0.4363 mg/L	8.114 ± 0.946	0.029 ± 0.005	0.024 ± 0.003[a]	1.121± 0.110	1.089 ± 0.103	1.409 ± 0.062	1.410 ± 0.060	1.940 ± 0.085
Satellite control	8.992 ± 1.451	0.029 ± 0.002	0.029 ± 0.001	1.047 ± 0.083	1.037 ± 0.098	1.426 ± 0.092	1.416 ± 0.079	2.016 ± 0.298
Satellite group	8.913 ± 1.003	0.031 ± 0.002	0.031 ± 0.001[a]	1.085 ± 0.118	1.172 ± 0.234	1.419 ± 0.132	1.412 ± 0.139	2.092 ± 0.143

[a] Statistically different from control ($p < 0.05$)

Table A6.4.3-5: Summary of absolute organ weight (g) – Female rats

Group/ Dose	Liver	Adrenal left	Adrenal right	Kidney left	Kidney right	Gonad left	Gonad right	Lungs
0 mg/L	6.002 ± 0.347	0.027 ± 0.004	0.024 ± 0.005	0.740 ± 0.071	0.758 ± 0.076	0.051 ± 0.007	0.050 ± 0.007	1.963 ± 0.123
0.1149 mg/L	6.627 ± 0.405[a]	0.032 ± 0.005[a]	0.031 ± 0.003[a]	0.783 ± 0.092	0.788 ± 0.105	0.056 ± 0.011	0.057 ± 0.011	2.015 ± 0.132
0.2201 mg/L	7.086 ± 0.597[a]	0.033 ± 0.002[a]	0.032 ± 0.002[a]	0.861 ± 0.075[a]	0.876 ± 0.077[a]	0.051 ± 0.010	0.047 ± 0.010	2.014 ± 0.143
0.4363 mg/L	6.208 ± 0.651	0.032 ± 0.007[a]	0.028 ± 0.006	0.830 ± 0.093	0.852 ± 0.078[a]	0.046 ± 0.009	0.043 ± 0.012	2.009 ± 0.130
Satellite control	7.364 ± 1.173	0.031 ± 0.002	0.031 ± 0.002	0.888 ± 0.097	0.882 ± 0.107	0.057 ± 0.007	0.055 ± 0.007	1.863 ± 0.281
Satellite group	6.669 ± 0.377	0.029 ± 0.002[a]	0.028 ± 0.001[a]	0.775 ± 0.070[a]	0.779 ± 0.042	0.054 ± 0.007	0.053 ± 0.005	1.909 ± 0.084

[a] Statistically different from control ($p < 0.05$)

Table A6.4.3-6: Summary of relative organ weight (%) – Male rats

Group/ Dose	Liver	Adrenal left	Adrenal right	Kidney left	Kidney right	Gonad left	Gonad right	Lungs
0 mg/L	3.115 ± 0.445	0.011 ± 0.002	0.011 ± 0.001	0.396 ± 0.052	0.395 ± 0.034	0.527 ± 0.040	0.520 ± 0.038	0.787 ± 0.050
0.1149 mg/L	3.622 ± 0.291[a]	0.011 ± 0.001	0.012 ± 0.001[a]	0.433 ± 0.050	0.436 ± 0.047	0.548 ± 0.034	0.558 ± 0.038	0.816 ± 0.098
0.2201 mg/L	3.314 ± 0.483	0.013 ± 0.001[a]	0.013 ± 0.001[a]	0.445 ± 0.047[a]	0.464 ± 0.060	0.584 ± 0.050[a]	0.558 ± 0.046	0.842 ± 0.068
0.4363 mg/L	3.349 ± 0.309	0.012 ± 0.002	0.010 ± 0.002	0.463 ± 0.032[a]	0.450 ± 0.032[a]	0.583 ± 0.042[a]	0.583 ± 0.032[a]	0.803 ± 0.047
Satellite control	3.084 ± 0.486	0.010 ± 0.001	0.010 ± 0.001	0.360 ± 0.037	0.356 ± 0.039	0.490 ± 0.041	0.486 ± 0.036	0.689 ± 0.077
Satellite group	3.222 ± 0.583	0.011 ± 0.002	0.011 ± 0.002	0.403 ± 0.092	0.436 ± 0.117	0.533 ± 0.124	0.512 ± 0.092	0.756 ± 0.123

[a] Statistically different from control ($p < 0.05$)

Table A6.4.3-7: Summary of relative organ weight (%) – Female rats

Group/ Dose	Liver	Adrenal left	Adrenal right	Kidney left	Kidney right	Gonad left	Gonad right	Lungs
0 mg/L	2.612 ± 0.223	0.012 ± 0.002	0.011 ± 0.002	0.323 ± 0.047	0.331 ± 0.047	0.022 ± 0.002	0.022 ± 0.003	0.854 ± 0.077
0.1149 mg/L	3.165 ± 0.264[a]	0.015 ± 0.002[a]	0.015 ± 0.001[a]	0.373 ± 0.040[a]	0.376 ± 0.046[a]	0.027 ± 0.005[a]	0.027 ± 0.005[a]	0.965 ± 0.110[a]
0.2201 mg/L	3.363 ± 0.339[a]	0.015 ± 0.000[a]	0.015 ± 0.002[a]	0.409 ± 0.041[a]	0.416 ± 0.047[a]	0.024 ± 0.005	0.022 ± 0.004	0.955 ± 0.078[a]
0.4363 mg/L	2.817 ± 0.347	0.014 ± 0.004[a]	0.012 ± 0.002[a]	0.378 ± 0.057[a]	0.387 ± 0.046[a]	0.021 ± 0.004	0.019 ± 0.006	0.913 ± 0.090
Satellite control	2.701 ± 0.565	0.011 ± 0.001	0.011 ± 0.001	0.324 ± 0.047	0.323 ± 0.054	0.021 ± 0.003	0.020 ± 0.003	0.681 ± 0.120
Satellite group 0.4363 mg/L	2.496 ± 0.267	0.011 ± 0.001	0.010 ± 0.001[a]	0.290 ± 0.034	0.291 ± 0.030	0.020 ± 0.003	0.020 ± 0.003	0.713 ± 0.054

[a] Statistically different from control ($p < 0.05$)

Section A6.5 Annex Point IIIA 6.5	Chronic toxicity	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	Please refer to IIIA, 6.7.	
Undertaking of intended data submission [ ]	Not relevant	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*Give date of action*	
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*	
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A6.6.1 **Genotoxicity *in vitro***
Annex Point IIA6.6.1 ***In-vitro* gene mutation study in bacteria**

Official use only

24 Reference

24.1	**Reference**	[redacted] (1999) *Salmonella Typhimurium* Reverse Mutation Assay of Permethrin Technical. Microbiology Section, Department of Toxicology, [redacted] unpublished report no.: 1588. Dates of experimental work: November 25, 1998 – December 14, 1998.
24.2	**Data protection**	Yes
24.2.1	Data owner	Tagros Chemicals India Ltd.
24.2.2	Companies with letter of access	Not applicable
24.2.3	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/IA.

Comment [T25]: Confidential
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25 Guidelines and Quality Assurance

25.1	**Guideline study**	Yes, the test method was based on OECD Guideline 471
25.2	**GLP**	Yes
25.3	**Deviations**	No

26 MATERIALS AND MethodS

26.1	**Test material**	As given in section 2 (Permethrin 40:60)
26.1.1	Lot/Batch number	PH 01
26.1.2	Specification	As given in section 2 (Permethrin 40:60)
26.1.2.1	Description	Light yellow colour, viscous liquid
26.1.2.2	Purity	92.50%
26.1.2.3	Stability	Not relevant (single dose only)

Section A6.6.1 Genotoxicity *in vitro*

Annex Point IIA6.6.1 ***In-vitro* gene mutation study in bacteria**

26.2	**Study Type**	Bacterial reverse mutation test
26.2.1	Organism/cell type	*Salmonella. typhimurium*: TA 1535, TA 1537, TA 98, TA 100, TA 102
26.2.2	Deficiencies / Proficiencies	Histidine deficient All strains tested carry a mutation in one of several genes, which govern the biosynthesis of histidine. In addition, they also contain: rfa mutation, which creates a partial loss of the bacterial cell wall lipopolysaccharide barrier that increases the permeability of cells to higher molecular weight compounds. Uvr B mutation, which increases the susceptibility of the bacteria to several classes of mutagens by decreasing the DNA excision repair ability. R-factor plasmid (strains TA98, TA100 and TA102), which increases further the sensitivity of the tester strains by enhancing error-prone repair.
26.2.3	Metabolic activation system	S9 mix S9 derived from the liver microsomal enzymes from male Sprague-Dawley rats that were injected with Aroclor 1254 at 500 mg/kg. S9 fraction was obtained from the Division of Microbiology, Defence Research and Development Establishment (DRDE), Gwalior, India. The S9 mix was prepared immediately before its use and contained the following components:

Components	Volume (mL)
H_2O	3.8
0.2 M NaH_2PO_4/K_2HPO_4, pH 7.4	5.0
1 M Glucose-6-phosphate	0.1
0.1 M NADP	0.4
$MgCl_2$/0.825 M KCl	0.2
S9	0.5
Total	10.0

Section A6.6.1 **Genotoxicity *in vitro***

Annex Point IIA6.6.1 ***In-vitro* gene mutation study in bacteria**

26.2.4	Positive controls	Activation (S9): TA 98, TA 100, TA 1535, TA 1537 and TA 1538: 2-aminofluorene (dose of 20 µg/plate). Non activation: TA 98: 4-Nitro-1, 2-phenylene diamin (20 µg/plate) and TA 1538 : cumene hydroperoxide (100 µg/plate) TA 100 and TA 1535: sodium azide (10 µg/plate) TA 1537: 9-aminoacridine (150 µg/plate) Vehicle control: DMSO
26.3	**Administration / Exposure; Application of test substance**	
26.3.1	Concentrations	Dose range finding: 500, 250, 125, 62.5 and 31.25 µg/plate. Mutagenicity assay: 3.125, 6.25, 12.5, 25 and 50 µg/plate.
26.3.2		**Cytotoxicity test:** The plates were incubated at 37^0C ± 1^0C for 48 or 72 hours and examined to assess the state of background bacterial growth. **Mutagenicity test:** A quantity of 2 ml of top agar was added to sterile test tubes. 500 µl of 5% S9 mix (for testing in the presence of S9), 100µl of the appropriately diluted Permethrin technical and 100 µl of standard bacterial suspension were added to the tubes and mixed. The mix was added to Minimal Glucose Agar plate and was allowed to solidify. Triplicate plates were used for each test concentration.
26.3.3	Pre-incubation time	No pre-incubation
26.3.4	Other modifications	None
26.4	**Examinations**	
26.4.1	Number of cells evaluated	Not applicable

27 Results and Discussion

Section A6.6.1 Genotoxicity *in vitro*

Annex Point IIA6.6.1 ***In-vitro* gene mutation study in bacteria**

27.1	**Genotoxicity**	
27.1.1	without metabolic activation	No
27.1.2	with metabolic activation	No
27.2	**Cytotoxicity**	Yes

At dose levels of 62.5, 125, 250 and 500 µg/plate, slight backgound lawn inhibition was observed in all five strains (both in the presence and absence of S9). No cytotoxic effects were noted at 31.25µg/plate.

28 Applicant's Summary and conclusion

28.1 Materials and methods

Salmonella typhimurium strains TA 98, TA 100, TA 102 TA 1535 and TA 1537 were exposed to Permethrin technical at the following dose levels 3.125 to 50 µg/plate, dissolved in DMSO.

This study was conducted according to OECD guideline 471 and is described under point 3 with no deviation.

28.2 Results and discussion

Permethrin technical was not cytotoxic to the bacterial strains used at 31.25 µg/plate.

In the absence of S9, the number of revertants did not significantly alter in any of the strains tested, at any of the dose levels, when compared to the negative control. Statistical analysis did not show any dose dependent increase. Results are summarised in Table A6.6.1-1.

In the presence of S9, there were slight variations in the number of revertants in all strains, at dose levels of 3.125 to 50 µg/plate, when compared to the negative control. However, the variations were not dose dependent and not statistically significant. Results are summarised in Table A6.6.1-2.

No statistically significant increase in the number of revertants was therefore seen at any test concentration, in any of the bacterial strains tested (in the presence and absence of S9).

28.3 Conclusion

Permethrin technical did not cause a positive response with any of the tester strains either in the presence or absence of microsomal enzymes. Under the conditions of the test, Permethrin technical was found to be non-mutagenic.

28.3.1 Reliability 1

Section A6.6.1	Genotoxicity *in vitro*
Annex Point IIA6.6.1	***In-vitro* gene mutation study in bacteria**
28.3.2 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	11th May 2009
Materials and Methods	The applicant's version is acceptable.
Results and discussion	The applicant's version is adopted.
Conclusion	As indicated by the applicant, Permethrin technical did not cause a positive response with any of the tester strains either in the presence or absence of microsomal enzymes. Under the conditions of the test, Permethrin technical was found to be non-mutagenic.
Reliability	1
Acceptability	Acceptable
Remarks	None
	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 A6.6.1-1: Mean plate counts in the absence of S9

Conc. of Permethrin technical (µg/plate)	His+ revertant colonies/plate (Mean ± SD)*				
	TA1537	**TA1535**	**TA98**	**TA100**	**TA102**
DMSO	20.33 ± 1.53	21.67 ± 2.52	23.67 ± 5.51	125.67 ± 5.51	264.33 ± 7.57
3.125	17.00 ± 2.65	16.00 ± 5.57	26.33 ± 3.22	131.33 ± 8.15	257.33 ± 32.72
6.25	18.67 ± 0.58	14.00 ± 2.65	25.33 ± 4.62	129.00 ± 7.81	245.33 ± 46.31
12.5	16.33 ± 2.31	13.33 ± 3.79	25.00 ± 5.29	124.67 ± 1.16	265.33 ± 31.00
25	15.67 ± 5.51	13.33 ± 0.58	24.33 ± 0.58	126.33 ± 2.31	222.33 ± 22.03
50	13.33 ± 2.52	13.67 ± 3.51	24.67 ± 2.31	121.67 ± 3.79	230.67 ± 11.06
Positive Controls					
9-aminoacridine (150 µg/plate)	65.67 ± 6.11	NA	NA	NA	NA
2-aminofluorene (20 µg/plate)	NA	NA	NA	NA	NA
Sodium azide (10 µg plate)	NA	4539.67 ± 644.30	NA	2267.67 ± 422.53	NA
4-nitro-1,2-phenylene diamin (20 µg/plate)	NA	NA	3942.67 ± 174.00	NA	NA
Cumene hydroperoxide (100 µg/plate)	NA	NA	NA	NA	1234.67 ± 66.53

* Mean and standard deviation of three replicates
NA: Not applicable

Table A6.6.1-2: Mean plate counts in the presence of S9 (5% v/v)

Conc. of Permethrin technical (µg/plate)	His+ revertant colonies/plate (Mean ± SD)*				
	TA1537	TA1535	TA98	TA100	TA102
DMSO	20.67 ± 1.53	15.67 ± 3.06	37.33 ± 3.06	145.00 ± 2.00	283.33 ± 21.78
3.125	23.00 ± 2.65	14.67 ± 1.53	35.67 ± 4.51	147.33 ± 1.53	231.33 ± 29.02
6.25	15.67 ± 3.51	15.33 ± 2.08	33.00 ± 3.00	144.33 ± 1.16	234.00 ± 22.27
12.5	17.00 ± 2.00	17.67 ± 2.31	34.00 ± 5.29	134.00 ± 5.57	280.67 ± 14.57
25	22.33 ± 1.53	16.00 ± 5.57	31.67 ± 8.08	142.00 ± 7.81	200.33 ± 22.12
50	18.33 ± 2.08	14.33 ± 1.16	31.67 ± 2.08	139.00 ± 8.66	237.00 ± 22.72
Positive Controls					
2-aminofluorene (20 µg/plate)	4134.33 ± 96.55	50.67 ± 11.37	5824.33 ± 317.15	2583.33 ± 20.03	783.33 ± 50.60

* Mean and standard deviation of three replicates
NA: Not applicable

Section A6.6.2 **Genotoxicity *in vitro***
Annex Point IIA6.6.2 ***In Vitro* cytogenicity study in mammalian cells**

Official use only

29 Reference

Reference	[redacted] (2003) *In Vitro* Mammalian Chromosome Aberration Test with Permethrin [redacted] unpublished report no.: 3352/02
	Dates of experimental work: July 8, 2002 – November 20, 2002.
Data protection	Yes
Data owner	Tagros Chemicals India Ltd.
Companies with letter of access	Not applicable
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/IA.

Comment [T26]: Confidential
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Guidelines and Quality Assurance

Guideline study	Yes, the test method was based on OECD Guideline 473
GLP	Yes
Deviations	No

MATERIALS AND MethodS

Test material	As given in section 2 (Permethrin 25:75)
Batch number	143
Specification	As given in section 2 (Permethrin 25:75)
Description	Yellow to pale brown coloured viscous liquid, tends to crystallise partly at room temperature with mild characteristic odour.
Purity	92.40 %
Stability	Not applicable

Section A6.6.2 Genotoxicity *in vitro*

Annex Point IIA6.6.2 ***In Vitro* cytogenicity study in mammalian cells**

Study Type	*In Vitro* Mammalian Chromosome Aberration Test.
Organism/cell type	Chinese hamster Ovary (CHO) CHO –K1 cell line (Ovary, Chinese hamster, Cricetulus griseus)
Source	[redacted]. The cells were shown to be sterile from mycoplasma contamination, with a modal chromosome number of 20.
Deficiencies / Proficiencies	Not applicable
Metabolic activation system	S9 mix S9 derived from the liver microsomal enzymes from male Wistar rats that were injected with Aroclor 1254 at 500 mg/kg. The S9 mix was prepared immediately before its use. The S9 mix was prepared immediately prior to use in the mutagenic assay, by mixing 1 part of S9 homogenate with 9 parts co-factor solution, kept on an ice bath and used within 1 hour. The co-factor solution contained NADP (4 mM), Glucose-6-phosphate (5 mM); Magnesium chloride (8 mM) and Potassium chloride (33 mM) as follows

Components	Preliminary cytotoxicity (mg)	Trial I (mg)	Trial II (mg)
NaH_2PO_4/K_2HPO_4, pH 7.4	10 mL	28 mL	37 mL
5 mM Glucose-6-phosphate	17	48	63
4 mM NADP	31	88	116
8 mM $MgCl_2$	16	46	60
33mM KCl	25	69	91

Positive control	Ethylmethanesulphonate at 600 µg/ml, in the absence of S9 Cyclophosphamide at 55 µg/ml, in the presence of S9
Administration / Exposure; Application of test substance	

Comment [T27]: Confidential

Formatted: Highlight

Section A6.6.2	Genotoxicity *in vitro*
Annex Point IIA6.6.2	*In Vitro* cytogenicity study in mammalian cells

Concentrations	Preliminary cytotoxicity test: 0, 20, 40, 80, 160, 320, 640, 1280 µg/ml. Trial I (Presence of S9) 0, 70, 210, 630 µg/ml Trial I (Absence of S9) 0, 40, 120, 360 µg/ml Trial II (Presence of S9) 0, 70, 210, 630, µg/ml Trial II (Absence of S9) 0, 15, 45, 135, µg/ml Vehicle control: DMSO
Way of application	Preliminary cytotoxicity test: Exponentially growing CHO-K1 cells were plated in F12 FBS10 at a density of approximately 5 x 10^5 cells/25 cm^2 flask and incubated for 23 hours. The target cells, in duplicate cultures, were exposed to the following concentrations of Permethrin technical - DMSO control, 20, 40, 80, 160, 320, 640, 1280 µg/ml. Three sets of each group were prepared. Set 1 (with S9) and Set II (without S9) were exposed for 3 hours. Set III (without S9) were exposed for 20 hours. In tests incorporating metabolic activation, S9 mix was added to give a final concentration of 10 % (v/v) in the test suspensions. After the treatment period, the cell monolayer was washed twice with PBS, refreshed with F12 FBS10 and incubated for approximately 17 hours. For evaluation of cytotoxicity, the cells from each flask were trypsinized, the cells suspended in F12 FBS10, replicates pooled and the cell counts determined separately using a hemocytometer. The effect of Permethrin technical on cell multiplication was estimated by expressing the number of cells in each treated culture as a percentage of the number in the DMSO control. Trial I & II - Chromosome aberration test Exponentially growing CHO-K1 cells were plated in F12 FBS10 at a density of approximately 10^6 cells in 75 cm^2 flask and incubated for 23 hours. The target cells were exposed to DMSO, the positive controls and 3 concentrations of Permethrin technical. The treatment medium consisted of 13 and 15 ml of F12 FBS10, for tests in the presence and absence of S9, respectively, and 300µl of Permethrin technical diluted to the appropriate concentration in DMSO. In tests incorporating metabolic activation, S9 mix was added to give a final concentration of 7.5 and 10% (v/v) in the test suspensions. For Trial I, cells were exposed to Permethrin technical for 3 hours; for Trial II, cells in the presence of S9 were exposed for 3 hours, cells in the absence of S9 were exposed for 19 hours. After the treatment period, the medium was

Section A6.6.2 **Genotoxicity *in vitro***

Annex Point IIA6.6.2 ***In Vitro* cytogenicity study in mammalian cells**

removed, the cell monolayer was washed with PBS and the flasks refreshed with F12 FBS10 and kept for incubation.

Colchicine was added to the culture medium approximately 1.75 hours before the required harvest time. At the end of the incubation period, mitotic cells were suspended in medium after trypsinization, centrifuged at 1000 rpm, suspended in potassium chloride and incubated for 10 minutes at room temperature. Cells were then centrifuged at 2000 rpm, the supernatant removed and cells resuspended in cold methanol: acetic acid fixative (3:1). Cells were again centrifuged at 2400 rpm, and the resuspension and centrifugation steps repeated twice. After the final resuspension, cells were incubated at room temperature for 10 minutes prior to slide preparation.

The cell suspension was dropped onto a clean chilled slide, flame dried and dried on a slide warmer at 40^0C.

Staining

The slides were stained with freshly prepared with 5% Giemsa stain for 20 minutes, rinsed, air dried, immersed in xylene and mounted with DPX. Slides were coded before evaluation.

Examinations

Number of cells evaluated

A total of 200 such metaphases from quintuplicate cultures were evaluated for each group.

The chromosome and chromatid aberrations observed were grouped into five categories – gaps, simple breaks, deletions, displacements and exchanges. Ring chromosomes were also recorded.

The microscope co-ordinates were recorded for each aberrant metaphase. The total number of metaphases showing one or more aberration, both including and excluding gaps, were calculated from a set of 200 metaphases for each group.

Further remarks

Concurrent cytotoxicity for all treated and control cultures were recorded soon after trypsinization, based on cell counts. At the time of chromosome preparation, the cell counts of mitotic cells from each group were determined using a hemocytometer.

The data analysed were the proportions of aberrant metaphases in each sample, both including and excluding gaps. The pooled data from each test concentration and positive control data were compared with DMSO control using one-tailed Fisher exact test. All analysis and comparisons were evaluated at 5% ($p \leq 0.05$) level.

Results and Discussion

Section A6.6.2 Genotoxicity *in vitro*

Annex Point IIA6.6.2 ***In Vitro* cytogenicity study in mammalian cells**

Genotoxicity	
without metabolic activation	No
with metabolic activation	No
Cytotoxicity	Yes

Preliminary cytotoxicity test:
Cytotoxicity (as demonstrated by >50% growth inhibition) was observed at 640 and 1280 µg/ml in the presence of S9, 160 and 640 and 1280 µg/ml in the absence of S9. In the extended test (Set III), exposed for 20 hours, cytotoxicity was observed at 160, 320, 640 and 1280 µg/ml.

Concurrent Cytotoxicity test:
In both trials, at the highest concentration tested, the reduction in cell growth was in the range of 51.75 to 53.19% over the DMSO control, both in the presence and absence of S9.

Applicant's Summary and conclusion

Materials and methods

CHO-KI cells were exposed to Permethrin technical at the following concentrations 0 to 630µg/ml in the presence of metabolic activation and 0 to 360 µg/ml in the absence of metabolic activation.

This study was conducted according to OECD guideline 473 and is described under point 3 with no deviations.

Results and discussion

Preliminary cytotoxicity test:
Cytotoxicity (as demonstrated by >50% growth inhibition) was observed at 640 and 1280 µg/ml in the presence of S9, 160 and 640 and 1280 µg/ml in the absence of S9. In the extended test (Set III), exposed for 20 hours, cytotoxicity was observed at 160, 320, 640 and 1280 µg/ml.

Concurrent Cytotoxicity test:
In both trials, at the highest concentration tested, the reduction in the cell growth was in the range of 51.75 to 53.19% over the DMSO control, both in the presence and absence of S9.

Results are summarised in Table A6.6.2-1.

In the absence of S9 mix:

Trial I:
There was no statistically significant increase in the incidence of

Section A6.6.2 **Genotoxicity *in vitro***

Annex Point IIA6.6.2 ***In Vitro* cytogenicity study in mammalian cells**

aberrant metaphases (both including and excluding gaps) observed at any test concentration, when compared with DMSO control.

Trial II:

There was no increase in the incidence of aberrant metaphases in any of the concentrations tested, when compared with the DMSO control.

In both trials, the positive control, ethylmethanesulphonate, caused a statistically significant increase in aberrant metaphases both including and excluding gaps. Results are summarised in Table A6.6.2-2 and A6.6.2-3.

In the presence of S9 mix:

Trial I:

There was no statistically significant increase in the incidence of aberrant metaphases (both including and excluding gaps) observed at any test concentration, when compared with DMSO control.

Trial II:

There was no statistically significant increase in the incidence of aberrant metaphases (both including and excluding gaps) observed at any test concentration, when compared with DMSO control.

In both trials, the positive control, cyclophosphamide, caused a statistically significant increase in aberrant metaphases, both including and excluding gaps. Results are summarised in Table A6.6.2-2 and A6.6.2-3.

There was no evidence of induction of chromosome aberrations by Permethrin technical, either in the presence or absence of S9, at any concentration tested.

Conclusion Under the conditions of the study, Permethrin technical does not have the potential to cause chromosome damage, either including or excluding gaps, in either the presence or absence of metabolic activation (S9).

Reliability 1

Deficiencies No

Section A6.6.2 **Genotoxicity *in vitro***

Annex Point IIA6.6.2 ***In Vitro* cytogenicity study in mammalian cells**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	15th June 2009
Materials and Methods	The applicant's version is acceptable.
Results and discussion	The applicant's version is adopted
Conclusion	Under the conditions of the study, Permethrin technical does not have the potential to cause chromosome damage, either including or excluding gaps, in either the presence or absence of metabolic activation (S9).
Reliability	1
Acceptability	Acceptable
Remarks	None
	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: Preliminary cytotoxicity test results with Permethrin technical on the growth of CHO cells

Test item Concentration (µg/ml)	With S9*		Without S9*		Without S9**	
	Cell Count (x 10^6/flask)	% Control	Cell Count (x 10^6/flask)	% Control	Cell Count (x 10^6/flask)	% Control
DMSO (0.1 ml)	3.03	100	3.53	100	3.34	100
20	2.79	92.08	3.37	95.47	2.17	64.97
40	2.89	95.38	2.28	64.59	2.10	62.87
80	1.85	61.06	1.80	50.99	2.14	64.07
160	1.94	64.03	1.70	48.16	1.28^{+}	38.32
320	1.55	51.16	1.77	50.14	1.12^{+}	33.53
640	1.32^{+}	43.56	1.52	43.06	1.17^{+}	35.03
1280	$0.98^{\$}$	32.34	$1.04^{\$}$	29.46	$0.79^{\$}$	23.65

* 3 hrs. treatment ** ~ 20 hrs treatment
$^{+}$: Damaged cells
$^{\$}$: Heavily damaged cells

Table A6.6.2-2: Trial I - Summary of results of *in vitro* mammalian chromosome aberration test with Permethrin technical in the presence and absence of S9

No. of MP's scored	With S9			Without S9		
	Test item conc. (µg/ml)	Total No. (%) of MP's with aberrations		Test item conc. (µg/ml)	Total No. (%) of MP's with aberrations	
		With Gaps	Without Gaps		With Gaps	Without Gaps
	DMSO (0.3 ml)	4 (2.0)	0	DMSO (0.3 ml)	5 (2.5)	3 (1.5)
200	70	1 (0.5)	0	40	1 (0.5)	0
200	210	2 (1.0)	0	120	5 (2.5)	0
200	630	3 (1.5)	1 (0.5)	360	4 (2.0)	2 (1.0)
200	CPA 55	166 $^{+}$ (83.0)	148^{+} (74.0)	EMS 600	114^{+} (57.0)	88^{+} (44.0)

MP: Metaphase $^{+}$ Significantly higher than control ($p \leq 0.05$) by Fisher exact test.

Table A6.6.2-3: Trial II - Summary of results of *in vitro* mammalian chromosome aberration test with Permethrin technical in the presence and absence of S9

No. of MP's scored	With S9			Without S9		
	Test item conc. (µg/ml)	Total No. (%) of MP's with aberrations		Test item conc. (µg/ml)	Total No. (%) of MP's with aberrations	
		With Gaps	Without Gaps		With Gaps	Without Gaps
	DMSO (0.3 ml)	1 (0.5)	0	DMSO (0.3 ml)	1 (0.5)	1 (0.5)
200	70	0	0	15	0	0
200	210	2 (1.0)	0	45	0	0
200	630	1 (0.5)	0	135	1 (0.5)	0
200	CPA 55	153^{+} (76.5)	116^{+} (58.0)	EMS 600	127^{+} (63.5)	91^{+} (45.5)

MP: Metaphase $^{+}$ Significantly higher than control ($p \leq 0.05$) by Fisher exact test.

Section A6.6.3 **Genotoxicity in vitro**
Annex Point IIA6.6.3 ***In vitro* mammalian cell gene mutation test**

Official use only

30 Reference

30.1	**Reference**	[redacted] (2002) *In vitro* Mammalian Cell Gene Mutation Test with Permethrin. Toxicology Department, [redacted] unpublished report no.: 3353/02. Dates of experimental work: June 24, 2002 to July 22, 2002
30.2	**Data protection**	Yes
30.2.1	Data owner	Tagros Chemicals India Ltd.
30.2.2	Companies with letter of access	Not applicable
30.2.3	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/IA.

Comment [T28]: Confidential
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31 Guidelines and Quality Assurance

31.1	**Guideline study**	Yes, the test method was based on OECD Guideline 476
31.2	**GLP**	Yes
31.3	**Deviations**	No

32 MATERIALS AND MethodS

32.1	**Test material**	As given in section 2 (Permethrin 25:75)
32.1.1	Lot/Batch number	143
32.1.2	Specification	As given in section 2 (Permethrin 25:75)
32.1.2.1	Description	Yellow to pale brown coloured viscous liquid, with a mild characteristic odour, which tends to crystallise partly at room temperature.
32.1.2.2	Purity	92.4%

Section A6.6.3 Genotoxicity in vitro

Annex Point IIA6.6.3 ***In vitro* mammalian cell gene mutation test**

32.1.2.3 Stability	Not relevant (single dose only)
32.2 Study Type	*In Vitro* mammalian cell gene mutation test
32.2.1 Organism/cell type	Chinese hamster Ovary (CHO) CHO-K1 cell line (Ovary, Chinese hamster, *Cricetulus griseus*)
32.2.2 Source	[redacted]
32.2.3 Deficiencies	Hypoxanthine-Guanine Phosphoribosyl-Transferase (HGPT) deficient
32.2.4 Metabolic activation system	S9 mix: batch no.: 14; protein content: 24.8 mg/ml S9 derived from the liver microsomal enzymes from male Wistar rats that were injected with Aroclor 1254 at 500 mg/kg. The S9 mix was prepared immediately before its use. The S9 mix was prepared immediately prior to use in the mutagenic assay, by mixing 1 part of S9 homogenate with 9 parts co-factor solution, kept on an ice bath and used within 1 hour. The co-factor solution contained NADP (4 mM), Glucose-6-phosphate (5 mM); Magnesium chloride (8 mM) and Potassium chloride (33 mM) as follows:

Components	Preliminary cytotoxicty (mg)	Trial I (mg)	Trial II (mg)
NaH_2PO_4/K_2HPO_4, pH 7.4	10 mL	5 mL	7 mL
5 mM Glucose-6-phosphate	17	9	12
4 mM NADP	31	16	22
8 mM $MgCl_2$/	16	8	11
33 mM KCl	25	12	17

32.2.5 Positive control	Ethylmethanesulphonate at 0.4 µg/ml, in the absence of S9 Benzo (a) pyrene at 3 µg/ml, in the presence of S9
32.3 Administration / Exposure; Application of test substance	

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Section A6.6.3 Genotoxicity in vitro

Annex Point IIA6.6.3 ***In vitro* mammalian cell gene mutation test**

32.3.1 Concentrations

Preliminary cytotoxicity test:
25, 50, 100, 200, 400, 800 µg/ml

Trial I (Presence of S9)
20, 60, 180 and 540 µg/ml

Trial I (Absence of S9)
25, 63, 156 and 391 µg/ml

Trial II (Presence of S9)
20, 60, 180 and 540 µg/ml

Trial II (Absence of S9)
25, 55, 151 and 416 µg/ml

Vehicle control: DMSO

32.3.2 Way of application

Cytotoxicity test:

Preliminary cytotoxicity test: Exponentially growing CHO-KI cells were plated in F12 FBS10 at a density of approximately 5 x 10^5 cells/25 cm^2 flask and incubated for 23 hours. The target cells, in duplicate cultures, were exposed to the following concentrations of Permethrin technical (DMSO control, 25, 50, 100, 200, 400 and 800 µg/ml) for 5 hours at 37±1°C. Cells were incubated in the presence and absence of metabolic activation. After the treatment period, the cells were washed with PBS, refilled with F12 FBS10 and incubated for approximately 17 hours.

Following preparation for evaluation, the effect of Permethrin technical on cell multiplication was estimated by expressing the number of cells in each treated culture as a percentage of the number in the DMSO control.

Mutation assay:

Exponentially growing CHO-KI cells were plated in F12 FBS10 at a density of approximately 5 x 10^5 cells/25 cm^2 flask and incubated for 24 hours. The target cells, in duplicate cultures were exposed to DMSO, the positive control and 4 concentrations of Permethrin technical for 5 hours at 37±1°C. After the treatment period, the cells were washed with PBS, cultured in F12 FBS10 and incubated for approximately 17 hours.

Cytotoxicity was expressed as an effect of the test item on cell multiplication by expressing the number of cells in each treated culture as a percentage of the number in the DMSO control.

For expression of the mutant phenotype, the cells from the pooled replicates were subcultured in F12 FBS5, in duplicate, at a density of approximately 10^6-cells/90 mm dish and incubated. Subculture at 2-3 days intervals was carried out for the 7-9 day expression period. After

Section A6.6.3 **Genotoxicity in vitro**
Annex Point IIA6.6.3 ***In vitro* mammalian cell gene mutation test**

this time, the mutant phenotype was selected.

For selection of the 6-Thioguanine (6TG) resistant phenotype, the replicates from controls and each treatment condition were pooled and replated, in quintuplicate, at a density of approximately 2 x 10^5 cells/90 mm dish in F12 FBS10 containing 20 µM 6TG and incubated for 10 days.

For cloning efficiency determination at the time of selection, 100-cells/25 cm^2 flasks was plated in triplicate in F12 FBS10 and incubated for 7 days.

32.3.3 Pre-incubation time: 23 hours

32.3.4 Other modifications: The colonies were stained with methylene blue and counted for both cloning efficiency and mutant selection, after 7 and 10 days of incubation respectively.

32.4 Examinations

32.4.1 Number of cells evaluated: 1 x 10^6 clonable cells

33 Results and Discussion

33.1 Genotoxicity

33.1.1 without metabolic activation: No

No significant increase, in trial I or II, in the frequencies of mutants compared to solvent control in the absence of metabolic activation at the tested concentrations.

33.1.2 with metabolic activation: No

No significant increase, in trial I or II, in the frequencies of mutants compared to the DMSO control in the presence of metabolic activation at the tested concentrations.

33.2 Cytotoxicity: Yes

Preliminary cytotoxicity test:

Growth inhibition: 64.55% and 67.25% at 800 µg/ml

Parallel cytotoxicity test:

In both trials (I and II),

Reduction in cell growth 37.33% and 60.6% at 540 µg/ml

Section A6.6.3 **Genotoxicity in vitro**

Annex Point IIA6.6.3 ***In vitro* mammalian cell gene mutation test**

34 Applicant's Summary and conclusion

34.1 Materials and methods

CHO-KI cells were exposed to Permethrin technical in DMSO at concentrations of 20 to 540 µg/mL in the absence of metabolic activation and at concentrations of 20 to 416 µg/mL in the presence of metabolic activation.

This study was conducted according to OECD guideline 476 and is described under point 3 with no deviations.

34.2 Results and discussion

Preliminary cytotoxicity test:

At the highest tested concentration, 800 µg/ml, there was evidence of growth inhibition by 64.55% and 67.25% over the DMSO control in the presence and absence of metabolic activation respectively.

Parallel cytotoxicity test:

In both trials (I and II), at the highest concentration tested (540 µg/ml), the reduction in the cell growth was in the range of 37.33% to 60.6% over the DMSO control, both in the presence and absence of metabolic activation respectively.

Permethrin technical did not cause a significant increase, in trial I or II, in the frequencies of mutants compared to the DMSO control in the presence of metabolic activation at the tested concentrations. Results are summarised in Tables A6.6.3-1 and A6.6.3-3. However, under similar conditions the positive control, Benzo (a) pyrene, induced a significant increase in the mutant frequency as compared with the solvent control.

Permethrin technical did not cause a significant increase, in trial I or II, in the frequencies of mutants compared to solvent control in the absence of metabolic activation at the tested concentrations. Results are summarised in Tables A6.6.3-2 and A6.6.3-4. However, under similar conditions the positive control, Ethylmethanesulphonate, induced a significant increase in the mutant frequency as compared with the solvent control.

34.3 Conclusion

Permethrin technical was not mutagenic under the test conditions of this study.

34.3.1 Reliability

1

34.3.2 Deficiencies

No

Section A6.6.3 **Genotoxicity in vitro**

Annex Point IIA6.6.3 ***In vitro* mammalian cell gene mutation test**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	16th June 2009
Materials and Methods	The applicants version is acceptable.
Results and discussion	The applicant's version is adopted.
Conclusion	Permethrin technical was not mutagenic under the test conditions of this study.
Reliability	1
Acceptability	Acceptable
Remarks	None
	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	

Section A6.6.3 Genotoxicity in vitro

Annex Point IIA6.6.3 ***In vitro*** **mammalian cell gene mutation test**

Table A6.6.3-1: Cloning data with Permethrin technical in the presence of metabolic activation (Trial I)

Test item concentration (µg/ml)	No. of colonies/R.M. plate					Total no. of mutants counted	No. of colonies/C.E. flask				ACE	Mutants/10^6 survivors
	1	2	3	4	5		1	2	3	Mean		
DMSO (0.1ml)	1	1	0	0	0	2	72	79	72	74	74	3
20	2	0	1	0	0	3	84	76	59	73	73	4
60	0	0	0	1	2	3	69	72	67	69	69	4
180	0	1	0	0	0	1	66	73	62	67	67	1
540	0	0	0	0	0	0	68	69	76	71	71	0
B (a) p 3	22	23	19	23	26	113	63	71	64	66	66	171+

R.M.= Restrictive Medium C.E.= Cloning Efficiency ACE = Absolute Cloning Efficiency

Mean, ACE and mutants/10^6 survivors values are rounded to the nearest whole number

+: Significantly higher than control by Dunnett's test

Table A6.6.3-2: Cloning data with Permethrin technical in the absence of metabolic activation (Trial I)

Test item concentration (µg/ml)	No. of colonies/R.M. plate					Total no. of mutants counted	No. of colonies/C.E. flask				ACE	Mutants/10^6 survivors
	1	2	3	4	5		1	2	3	Mean		
DMSO (0.1ml)	0	3	0	1	1	5	86	85	79	83	83	6
25	1	1	1	0	0	3	81	82	85	83	83	4
63	1	1	1	1	0	4	60	68	78	69	69	6
156	0	1	0	1	0	2	87	67	79	78	78	3
391	1	0	0	2	1	4	68	66	72	69	69	6
EMS 0.4 µg/ml	73	65	73	64	58	333	71	61	74	69	69	483*

R.M.= Restrictive Medium C.E.= Cloning Efficiency ACE = Absolute Cloning Efficiency

Mean, ACE and mutants/10^6 survivors values are rounded to the nearest whole number

+: Significantly higher than control by Dunnett's test

Table A6.6.3-3: Cloning data with Permethrin technical in the presence of metabolic activation (Trial II)

Test item concentration (µg/ml)	No. of colonies/R.M. plate					Total no. of mutants counted	No. of colonies/C.E. flask				ACE	Mutants/10^6 survivors
	1	2	3	4	5		1	2	3	Mean		
DMSO (0.1ml)	1	2	1	0	0	4	82	79	86	82	82	5
20	1	1	0	0	1	3	76	81	73	77	77	4
60	0	0	0	1	1	2	74	72	69	72	72	3
180	1	0	1	0	1	3	62	68	60	63	63	5
540	0	0	0	0	1	1	59	63	65	62	62	2
B (a) p 3	26	22	23	20	18	109	66	64	58	63	63	173^+

R.M.= Restrictive Medium C.E.= Cloning Efficiency ACE = Absolute Cloning Efficiency

Mean, ACE and mutants/10^6 survivors values are rounded to the nearest whole number

+: Significantly higher than control by Dunnett's test

Table A6.6.3-4: Cloning data with Permethrin technical in the absence of metabolic activation (Trial II)

Test item concentration (µg/ml)	No. of colonies/R.M. plate					Total no. of mutants counted	No. of colonies/C.E. flask				ACE	Mutants/10^6 survivors
	1	2	3	4	5		1	2	3	Mean		
DMSO (0.1ml)	0	0	1	2	0	3	76	81	73	77	77	4
20	1	1	0	0	0	2	79	82	84	82	82	2
55	0	0	2	1	0	3	72	66	73	70	70	4
151	1	1	1	0	0	3	67	63	69	66	66	5
416	0	0	1	1	0	2	62	60	79	67	67	3
Ems 0.4 µg/ml	66	56	52	79	72	325	55	63	69	62	62	524+

R.M.= Restrictive Medium C.E.= Cloning Efficiency ACE = Absolute Cloning Efficiency

Mean, ACE and mutants/10^6 survivors values are rounded to the nearest whole number

+: Significantly higher than control by Dunnett's test

Section A6.6.3 **Genotoxicity in vitro**
Annex Point IIA6.6.3/02 ***In vitro* mammalian DNA damage**

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35 Reference

35.1	**Reference**	Ü. Ündeğer and N. Başaran. Effects of pesticides on human peripheral lymphocytes in vitro: induction of DNA damage. Arch. Toxicol (2005) 79: 169-176.
35.2	**Data protection**	No
35.2.1	Data owner	N/A
35.2.2	Companies with letter of access	N/A
35.2.3	Criteria for data protection	None

36 Guidelines and Quality Assurance

36.1	**Guideline study**	No, literature data
36.2	**GLP**	No, peer reviewed publication
36.3	**Deviations**	N/A

37 MATERIALS AND MethodS

37.1	**Test material**	Permethrin,
37.1.1	Lot/Batch number	N/A, supplied by Chinoin-Sanofi (Budapest, Hungary)
37.1.2	Specification	cis:trans ratio 39.4:60.6
37.1.2.1	Description	Not available
37.1.2.2	Purity	98.2%

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Section A6.6.3	Genotoxicity in vitro
Annex Point IIA6.6.3/02	*In vitro* mammalian DNA damage

37.1.2.3	Stability	Not relevant (single dose only)
37.2	**Study Type**	*In Vitro* DNA damage assay, Comet assay
37.2.1	Organism/cell type	Human peripheral lymphocytes
37.2.2	Source	From heparinized whole blood collected by venipuncture from one 30-year-old non-smoking female donor not exposed to radiation or drugs.
37.2.3	Deficiencies	N/A
37.2.4	Metabolic activation system	N/A
37.2.5	Positive control	H_2O_2
37.3	**Administration / Exposure; Application of test substance**	
37.3.1	Concentrations	10 µg/ml (0.03 mM), 50 µg/ml (0.13 mM), 100 µg/ml (0.26 mM) and 200 µg/ml (0.51 mM).
37.3.2	Way of application	Incubation for 0.5 h at 37°C. Cell concentrations were approximately $2x10^5$/ml. Test substance was dissolved in DMSO (max. solvent concentration 1 % in the culture medium).
37.3.3	Pre-incubation time	N/A
37.3.4	Other modifications	N/A.
37.4	**Examinations**	
37.4.1	Number of cells evaluated	Images of 100 randomly selected lymphocytes, i.e. 50 cells from each of two replicate slides, were analysed from each sample and tail length, tail intensity and tail moment were measured (measure for DNA breakage)

38 Results and Discussion

38.1	**Genotoxicity**	
38.1.1	without metabolic activation	Some Tail length significantly increased at 50 and 200 µg/ml. Tail intensity significantly increased at 10 and 200 µg/ml and tail moment at 200 µg/ml.

Section A6.6.3	Genotoxicity in vitro
Annex Point IIA6.6.3/02	*In vitro* mammalian DNA damage
38.1.2 with metabolic activation	N/A
38.2 Cytotoxicity	N/A

39 Applicant's Summary and conclusion

39.1 Materials and methods	Comet assay, using different active substances in a comparative research oriented paper.
39.2 Results and discussion	Although there are some significant increases in tail length, moment and intensity, the information is considered limited and inconsistent by the authors.
39.3 Conclusion	Results not useful for the evaluation of genotoxicity of permethrin
39.3.1 Reliability	3
39.3.2 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	16/01/12
Materials and Methods	Applicants version is acceptable
Results and discussion	Applicants version is acceptable
Conclusion	The authors find that permehtrin increases DHA damage in relation to dose and conclude that the comet assay is a highly sensitive method for detection DNA damage by pesticides. This as at odds with the applicants conclusion. The RMS considers the finding interesting and the quality of the study acceptable. It is however, difficult to place a non-guideline, non-GLP study in the context of a complete and thorough genotox pack.
Reliability	2
Acceptability	Acceptable The study is not GLP and does not follow an established guideline. However, it is of acceptable quality.
Remarks	The statistical methods employed or the assay limitations are not well described.

Section A6.6.3 Genotoxicity in vitro

Annex Point IIA6.6.3/02 ***In vitro* mammalian DNA damage**

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 1 DNA damage in human peripheral lymphocytes treated with dimethoate, methyl parathion, propoxur, pirimicarb, cypermethrin and permethrin compared with H_2O_2-treated positive control cells and DMSO-treated negative control cells. Data represent mean values (±SEM) of tail length, tail intensity and tail moment of the alkaline comet assay and refer to 300 scores/concentration (100 scores/experiment, three experiments)

Treatment group		Comet assay parameter		
Compound	Concentration	Tail length	Tail intensity	Tail moment
Negative (solvent) control	10 Il/ml DMSO	2.14 ± 0.08	3.87 ± 0.48	0.06 ± 0.01
Positive control	100 IM H_2O_2	6.24 ± 0.16***	26.61 ± 1.49***	0.85 ± 0.06***
Dimethoate	10 Ig/ml (0.04 mM)	4.55 ± 0.35***	8.19 ± 2.07*	0.19 ± 0.04
	50 Ig/ml (0.02mM)	3.80 ± 0.60***	7.64 ± 1.42*	0.17 ± 0.03
	100 Ig/ml (0.44mM)	3.11 ± 0.19*	12.33 ± 0.38***	0.24 ± 0.01*
	200 Ig/ml (0.87mM)	6.14 ± 0.58***	18.30 ± 2.05***	0.55 ± 0.09***
Methyl parathion	101g/ml (0.04mM)	4.80 ± 0.07***	6.90 ± 2.19	0.20 ± 0.03
	501g/ml (0.02mM)	4.13 ± 0.60***	6.20 ± 0.51	0.15 ± 0.02
	100 Ig/ml (0.38 mM)	2.93 ± 0.17	10.66 ± 1.18**	0.22 ± 0.02*
	200 Ig/ml (0.76 mM)	7.55 ± 0.39***	24.01 ± 2.17***	0.78 ± 0.09***
Propoxur	10 Ig/ml (0.05 mM)	3.73 ± 0.52**	6.07 ± 1.28	0.12 ± 0.03
	50 Ig/ml (0.24 mM)	3.26 ± 0.33*	10.53 ± 2.24**	0.20 ± 0.05*
	100 Ig/ml (0.48 mM)	4.23 ± 1.24***	8.56 ± 3.10*	0.23 ± 0.13*
	200 Ig/ml (0.96 mM)	5.41 ± 0.19***	13.81 ± 1.10***	0.35 ± 0.03***
Pirimicarb	10 Ig/ml (0.04 mM)	2.63 ± 0.22	8.56 ± 1.09*	0.16 ± 0.02
	501g/ml (0.21 mM)	2.42 ± 0.34	6.10 ± 0.92	0.11 ± 0.04
	100 Ig/ml (0.42 mM)	2.82 ± 0.17	5.42 ± 0.38	0.10 ± 0.01
	200 Ig/ml (0.84 mM)	4.98 ± 0.32***	12.47 ± 0.51***	0.28 ± 0.01**
Cypermethrin	10 Ig/ml (0.02 mM)	2.83 ± 0.26	11.02 ± 0.73***	0.19 ± 0.01
	50 Ig/ml (0.12 mM)	3.06 ± 0.35*	6.10 ± 1.29	0.13 ± 0.04
	100 Ig/ml (0.24 mM)	2.81 ± 0.30	5.98 ± 1.00	0.11 ± 0.02
	200 Ig/ml (0.48 mM)	4.10 ± 0.74***	9.47 ± 0.50**	0.20 ± 0.01*
Permethrin	10 Ig/ml (0.03 mM)	2.63 ± 0.09	8.82 ± 0.60*	0.16 ± 0.02
	50 Ig/ml (0.13 mM)	3.09 ± 0.44*	7.43 ± 1.47	0.14 ± 0.03
	100 Ig/ml (0.26 mM)	3.00 ± 0.09	6.62 ± 0.76	0.15 ± 0.02
	200 Ig/ml (0.51 mM)	4.17 ± 0.26***	10.02 ± 1.02**	0.21 ± 0.02*

*p < 0.5; **p < 0.01; ***p < 0.001, significance of DNA damage in dimethoate, methyl parathion, propoxur, pirimicarb, cypermethrin, permethrin and H_2O_2 treated peripheral lymphocytes compared with negative control cells

Section A6.6.3 **Genotoxicity in vitro**

Annex Point IIA6.6.3/03 ***In vitro* mammalian DNA damage**

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~~1~~40 Reference

~~1.1~~40.1 **Reference**	M. Tisch, P. Schmezer, M. Faulde, A. Groh and H. Maier. Genotoxicity studies on permethrin, DEET and diazinon in primary human nasal mucosal cells. Eur. Arch. Otorhinolaryngol (2002) 259: 150-153	Formatted: Bullets and Numbering
~~1.2~~40.2 **Data protection**	No	Formatted: Bullets and Numbering
~~1.2.1~~40.2.1 Data owner	N/A	Formatted: Bullets and Numbering
~~1.2.2~~40.2.2 Companies with letter of access	N/A	Formatted: Bullets and Numbering
~~1.2.3~~40.2.3 Criteria for data protection	None	Formatted: Bullets and Numbering

~~2~~41 Guidelines and Quality Assurance

~~2.1~~41.1 **Guideline study**	No, literature data	Formatted: Bullets and Numbering
~~2.2~~41.2 **GLP**	No, peer reviewed publication	Formatted: Bullets and Numbering
~~2.3~~41.3 **Deviations**	N/A	Formatted: Bullets and Numbering

~~3~~42 MATERIALS AND MethodS

~~3.1~~42.1 **Test material**	Permethrin,	Formatted: Bullets and Numbering
~~3.1.1~~42.1.1 Lot/Batch number	N/A, (Hamburg, Germany)	Formatted: Bullets and Numbering
~~3.1.2~~42.1.2 Specification	cis:trans ratio not given	Formatted: Bullets and Numbering
~~3.1.2.1~~42.1.2.1 Description	Not available	Formatted: Bullets and Numbering
~~3.1.2.2~~42.1.2.2 Purity	99.5%	Formatted: Bullets and Numbering

Section A6.6.3 **Annex Point IIA6.6.3/03**	**Genotoxicity in vitro** ***In vitro* mammalian DNA damage**	Formatted Table
~~3.1.2.3~~42.1.2.3 Stability	Not given although incubation of 1 hour	Formatted: Bullets and Numbering
~~3.2~~42.2 Study Type	*In Vitro* DNA damage assay, Comet assay	Formatted: Bullets and Numbering
~~3.2.1~~42.2.1 Organism/cell type	Human nasal mucosal cells	Formatted: Bullets and Numbering
~~3.2.2~~42.2.2 Source	Biopsies from 21 patients (16 male, 5 female, median age: 35.4 years) who underwent nasal surgery. Biopsies were taken from the middle and inferior turbinate.	Formatted: Bullets and Numbering
~~3.2.3~~42.2.3 Deficiencies	N/A	Formatted: Bullets and Numbering
~~3.2.4~~42.2.4 Metabolic activation system	N/A	Formatted: Bullets and Numbering
~~3.2.5~~42.2.5 Positive control	MNNG (*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine)	Formatted: Bullets and Numbering
~~3.3~~42.3 Administration / Exposure; Application of test substance		Formatted: Bullets and Numbering
~~3.3.1~~42.3.1 Concentrations	0.5 to 1.0 mM	Formatted: Bullets and Numbering
~~3.3.2~~42.3.2 Way of application	Incubation for 1 h at 37°C. Dissolved in DMSO.	Formatted: Bullets and Numbering
~~3.3.3~~42.3.3 Pre-incubation time	N/A	Formatted: Bullets and Numbering
~~3.3.4~~42.3.4 Other modifications	N/A.	Formatted: Bullets and Numbering
~~3.4~~42.4 Examinations		Formatted: Bullets and Numbering
~~3.4.1~~42.4.1 Number of cells evaluated	51 randomly selected DNA spots per slide (3 slides per concentration). Tail length measures (measure for DNA breakage)	Formatted: Bullets and Numbering
	43 Results and Discussion	Formatted: Bullets and Numbering
~~4.1~~43.1 Genotoxicity		Formatted: Bullets and Numbering
~~4.1.1~~43.1.1 without metabolic activation	A dose-dependent increase in genotoxic effects	Formatted: Bullets and Numbering
~~4.1.2~~43.1.2 with metabolic activation	N/A	Formatted: Bullets and Numbering

Section A6.6.3 **Annex Point IIA6.6.3/03**	**Genotoxicity in vitro** ***In vitro* mammalian DNA damage**
~~4.2~~43.2 Cytotoxicity	No cytotoxicity at the tested concentration range (0.5 to 1.0 mM).

~~5~~44 Applicant's Summary and conclusion

~~5.1~~44.1 Materials and methods	Comet assay, using different active substances in a comparative research oriented paper.
~~5.2~~44.2 Results and discussion	Although a significant concentration dependend genotoxic response was observed, the relevance of the results is doubtful in view of the assessment of permethrin as insufficient information is available on the test material. The concentrations tested have no link to exposure and the cells used in the tests are difficult to place in the regulatory toxicology approach.
~~5.3~~44.3 Conclusion	Results not useful for the evaluation of genotoxicity of permethrin
~~5.3.1~~44.3.1 Reliability	3
~~5.3.2~~44.3.2 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	~~*Give date of action*~~*18/1/2012*
Materials and Methods	*Accept applications conclusion*~~*State if the applicants version is acceptable or indicate relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*~~
Results and discussion	The source of the cells used for the assay is not clear. In addition cell from different biopsies appeared to produce different result.The batch number is not stated. The isomeric ratio of permethrin is not stated. ~~Adopt applicant's version include revised version. If necessary, discuss relevant deviations from applicant view referring to the (sub)heading numbers~~The study is non-guideline and non-GLP and the source of the cells is unusual. The term undamaged cells is not clearly defined. The study is supplementary information only.
Conclusion	Other conclusions: Suitable as supplementary information ~~(Adopt applicant's version or include revised version)~~

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Section A6.6.3	**Genotoxicity in vitro**
Annex Point IIA6.6.3/03	***In vitro* mammalian DNA damage**
Reliability	~~*Based on the assessment of materials and methods include appropriate reliability indicator*~~3
Acceptability	una~~a~~cceptable ~~/ not acceptable~~ *(give reasons if necessary, e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat is necessary.)*
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 1 Percentage of undamaged cells after treatment with permethrin, diazinon, DEET and MNNG (mean ± SD)

Concentration (mM)	Middle turbinate % undamaged cells	Inferior turbinate % undamaged cells
SC (solvent control)	89.6 ± 5.7	92.4 ± 4.6
Permethrin 0.5	46.8 ± 7.4	58.2 ± 6.9
0.75	37.9 ± 4.6	44.3 ± 5.4
1.0	9.5 ± 5.6	28.3 ± 4.2
DEET 0.5	51.4 ± 4.6	65.4 ± 6.2
0.75	36.3 ± 3.4	48.3 ± 5.5
1.0	20.4 ± 5.2	28.3 ± 6.3
Diazinon 0.5	49.4 ± 4.5	62.3 ± 5.8
0.75	32.4 ± 4.8	46.7 ± 3.5
1.0	16.8 ± 5.8	32.3 ± 6.9
MNNG 0.5	5.5 ± 4.5	18.4 ± 6.9
0.75	0	6.2 ± 3.7
1.0	0	0

Section A6.6.3 **Genotoxicity in vitro**

Annex Point IIA6.6.3/01 ***In vivo* mammalian DNA damage**

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~~1~~45 Reference

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~~1.1~~45.1 **Reference**	R. Gabbianelli, C. Nasuti, G. Falcioni and F. Cantalamessa. Lymphocyte DNA damage in rats exposed to pyrethroids: effect of supplementation with Vitamins E and C. Toxicology 203 (2004) 17-26
~~1.2~~45.2 **Data protection**	No
~~1.2.1~~45.2.1 Data owner	N/A
~~1.2.2~~45.2.2 Companies with letter of access	N/A
~~1.2.3~~45.2.3 Criteria for data protection	None

~~2~~46 Guidelines and Quality Assurance

~~2.1~~46.1 **Guideline study**	No, literature data
~~2.2~~46.2 **GLP**	No, peer reviewed publication
~~2.3~~46.3 **Deviations**	N/A

~~3~~47 MATERIALS AND MethodS

~~3.1~~47.1 **Test material**	Permethrin,
~~3.1.1~~47.1.1 Lot/Batch number	N/A, (Activa, Milan, Italy)
~~3.1.2~~47.1.2 Specification	cis:trans ratio 25:75
~~3.1.2.1~~47.1.2.1 Description	Not available
~~3.1.2.2~~47.1.2.2 Purity	94.0%

Section A6.6.3	**Genotoxicity in vitro**
Annex Point IIA6.6.3/01	***In vivo* mammalian DNA damage**
~~3.1.2.3~~47.1.2.3 Stability	Not given (daily oral treatment)
~~3.2~~47.2 **Study Type**	*In Vivo* DNA damage assay, Comet assay
~~3.2.1~~47.2.1 Organism/cell type	Rat peripheral blood lymphocytes
~~3.2.2~~47.2.2 Source	Male Wistar rats [redacted]
~~3.2.3~~47.2.3 Deficiencies	N/A
~~3.2.4~~47.2.4 Metabolic activation system	N/A
~~3.2.5~~47.2.5 Positive control	None
~~3.3~~47.3 **Administration / Exposure; Application of test substance**	
~~3.3.1~~47.3.1 Concentrations	High dose 150 mg/kg bodyweight (1/10 of LD_{50}), Low dose 15 mg/kg (1/100 of LD_{50})
~~3.3.2~~47.3.2 Way of application	By intragastric tube
~~3.3.3~~47.3.3 Pre-incubation time	Rats (n=30 per dose) were treated for 60 days
~~3.3.4~~47.3.4 Other modifications	Another group of 15 rats were dosed for 22 days with 300 mg/kg bodyweight permethrin in corn oil ± Vitamin E (200 mg/kg bw/day) or Vitamin C (200 mg/kg bw/day)
~~3.4~~47.4 **Examinations**	
~~3.4.1~~47.4.1 Number of cells evaluated	N/A. Tail length, intensity and moment were measured (measure for DNA breakage)

48 Results and Discussion

~~4.1~~48.1 **Genotoxicity**	
~~4.1.1~~48.1.1 without metabolic activation	No effects at the low (15 mg/kg/day) dose. Significant increase at the high (150 mg/kg/day) dose for the 3 parameters (tail length, intensity and moment). The administration of 300 mg/kg/day during 22 days did not change the Comet assay parameters.
~~4.1.2~~48.1.2 with metabolic activation	N/A

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Section A6.6.3	**Genotoxicity in vitro**
Annex Point IIA6.6.3/01	***In vivo* mammalian DNA damage**
~~4.2~~48.2 **Cytotoxicity**	Not evaluated.

~~5~~49 Applicant's Summary and conclusion

~~5.1~~49.1 **Materials and methods**	Rats were treated with high (150 mg/kg/day) and low (15 mg/kg/day) dose permethrin (via intragastric tube) during 60 days. Lymphocytes were prepared and tail length, intensity and moment measured and compared to a negative control. A higher dose (300 mg/kg/day) administered for 22 days was also used alone or in combination with either Vitamin E or C.
~~5.2~~49.2 **Results and discussion**	A significant genotoxic response was observed (all 3 parameters) in the high dose group only (150 mg/kg/day). The additional experiment with 300 mg/kg/day for 22 days did not show a genotoxic response. The concentrations tested are excessive in comparison to expected exposure values and therefore the use and validity of this data is questionable in the scope of this BPD evaluation.
~~5.3~~49.3 **Conclusion**	Results not useful for the evaluation of genotoxicity of permethrin
~~5.3.1~~49.3.1 Reliability	3
~~5.3.2~~49.3.2 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	~~*Give date of action*~~*18/01/2012*
Materials and Methods	*Applicants version is acceptable*~~*State if the applicants version is acceptable or indicate relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*~~
Results and discussion	The concentrations tested are excessive in comparison to expected exposure. However, this does not nullify concern regarding the results of this study in the context of the mutagenic hazard.~~*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers*~~

Section A6.6.3 Genotoxicity in vitro

Annex Point IIA6.6.3/01 ***In vivo* mammalian DNA damage**

Conclusion	Other conclusions: The paper is heavily focused on the effect of active oxygen species in pyrethroid toxicity and suggested this is the cause of the comet results. Vitamins E and C are part of the experimental procedures. The companies assertion that the results are not relevant are not supported by the RMS. It is however, difficult to place a non-guideline, non-GLP study in the context of a complete and thorough genotox pack. ~~Other conclusions:~~ ~~*(Adopt applicant's version or include revised version)*~~
Reliability	~~*Based on the assessment of materials and methods include appropriate reliability indicator*~~2
Acceptability	Aacceptable ~~/ not acceptable~~ ~~*(give reasons if necessary, e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat is necessary.)*~~
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	

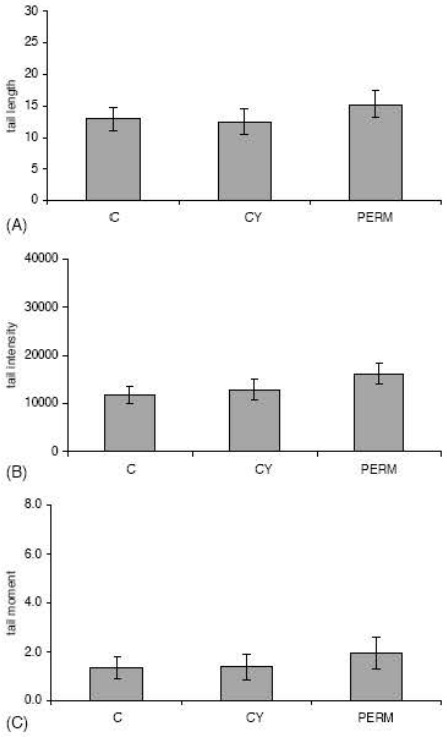

Fig. 3. Observed distributions of comet parameter tail length (A), tail intensity (B), and tail moment (C) in white blood cells from rat treated with low doses of pyrethroids (2.5 and 15 mg/kg body weight/day for CY and PERM, respectively). Data (at least 150 scores/sample) are mean values • S.E.M. of 12 samples (n • 4 rats for each group, three samples from each animal were used).

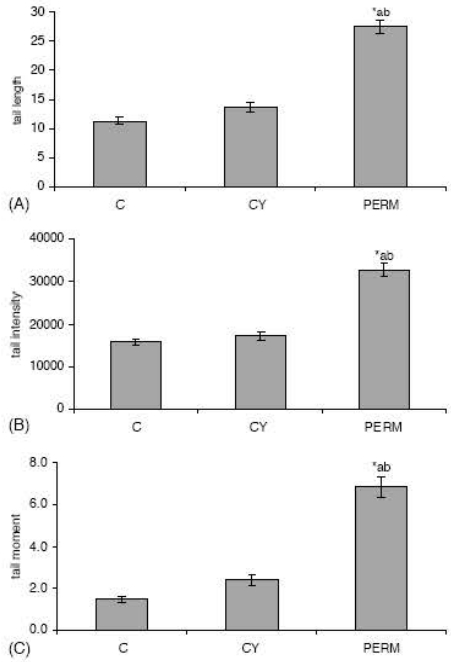

Fig. 4. Observed distributions of comet parameter tail length (A), tail intensity (B), and tail moment (C) in white blood cells from rat treated with high doses of pyrethroids (25 mg/kg body weight/day for CY and 150 mg/kg body weight/day for PERM). Data (at least 150 scores/sample) are mean values • S.E.M. of 12 samples (n • 4 rats for each group, three samples from each animal were used). [a] $P < 0.05$ compared to control group. [b] $P < 0.05$ compared to CY group.

Section A6.6.3 **Genotoxicity in vitro**

Annex Point IIA6.6.3/04 ***In vivo* mammalian DNA damage**

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~~1~~50 Reference

~~1.1~~50.1 **Reference**	M.L. Falcioni, C. Nasuti, C. Bergamini, R. Fato, G. Lenaz and R. Gabbianelli. The primary role of glutathione against nuclear DNA damage of striatum induced by permethrin in rats. Neuroscience 168 (2010) 2-10
~~1.2~~50.2 **Data protection**	No
~~1.2.1~~50.2.1 Data owner	N/A
~~1.2.2~~50.2.2 Companies with letter of access	N/A
~~1.2.3~~50.2.3 Criteria for data protection	None

~~2~~51 Guidelines and Quality Assurance

~~2.1~~51.1 **Guideline study**	No, literature data
~~2.2~~51.2 GLP	No, peer reviewed publication
~~2.3~~51.3 **Deviations**	N/A

~~3~~52 MATERIALS AND MethodS

~~3.1~~52.1 **Test material**	Permethrin,
~~3.1.1~~52.1.1 Lot/Batch number	N/A, (Activa, Milan, Italy)
~~3.1.2~~52.1.2 Specification	„technical grade“
~~3.1.2.1~~52.1.2.1 Description	Not available
~~3.1.2.2~~52.1.2.2 Purity	Not specified

Section A6.6.3 **Annex Point IIA6.6.3/04**	**Genotoxicity in vitro** ***In vivo* mammalian DNA damage**
~~3.1.2.3~~52.1.2.3 Stability	Not given
~~3.2~~52.2 **Study Type**	*In Vivo* DNA damage assay, Comet assay
~~3.2.1~~52.2.1 Organism/cell type	Rat striatum cells
~~3.2.2~~52.2.2 Source	Male Wistar rats [redacted] 120-130 g and about 5 weeks old.
~~3.2.3~~52.2.3 Deficiencies	N/A
~~3.2.4~~52.2.4 Metabolic activation system	N/A
~~3.2.5~~52.2.5 Positive control	None
~~3.3~~52.3 **Administration / Exposure; Application of test substance**	
~~3.3.1~~52.3.1 Concentrations	150 mg/kg bodyweight/day (1/10 of LD_{50}),
~~3.3.2~~52.3.2 Way of application	Pretreatment: orally, by intragastric tube
~~3.3.3~~52.3.3 Pre-incubation time	Rats (n=14) were treated for 60 days
~~3.3.4~~52.3.4 Other modifications	Another group of 8 rats received in addition to permethrin Vitamin E (280 mg/kg/day) and another group of 8 rats in addition to permethrin and Vitamin E Q_{10} (10 mg/kg). The negative control group (n=8) received 5 ml/kg bodyweight of corn oil for 60 days by intragastric tube.
~~3.4~~52.4 **Examinations**	
~~3.4.1~~52.4.1 Number of cells evaluated	N/A. % Tail DNA was used to quantify the DNA damage.
	~~4~~53 Results and Discussion
~~4.1~~53.1 **Genotoxicity**	
~~4.1.1~~53.1.1 without metabolic activation	The percentage tail DNA was significantly increased following permethrin treatment. Vitamin E supplementation maintained the % tail DNA as in the control and the simultaneous presence of coenzyme Q_{10} further reduced DNA damage to a value lower than the control

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