Section A6.6.3	Genotoxicity in vitro
Annex Point IIA6.6.3/04	*In vivo* mammalian DNA damage
~~4.1.2~~53.1.2 with metabolic activation	N/A
~~4.2~~53.2 Cytotoxicity	Not evaluated. General findings in the rats: no significant differences observed in body weight of treated versus control rats.

54 Applicant's Summary and conclusion

~~5.1~~54.1 Materials and methods	Rats were treated with 150 mg/kg/day permethrin (via intragastric tube) during 60 days. Striatum cells were prepared and % tail DNA was determined and compared to a negative control. In 2 other groups rats were also treated with either Vitamin E alone or in combination with Q_{10}.
~~5.2~~54.2 Results and discussion	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 This publication is trying to explain the mode of genotoxic action of permethrin in striatum cells from rats. The results are in line with another paper (Lymphocyte DNA damage in rats exposed to pyrethroids: effect of supplementation with Vitamin E and C. R. Gabbianelli et al., 2004). As the cells studied are not commonly used in regulatory toxicology studies, the relevance of this paper for the evaluation of the genotoxicity of permethrin within the scope of the current review is limited.
~~5.3~~54.3 Conclusion	Results not useful for the evaluation of genotoxicity of permethrin
~~5.3.1~~54.3.1 Reliability	3
~~5.3.2~~54.3.2 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*~~*19/1/2012*
Materials and Methods	~~*State if the applicants version is acceptable or indicate relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion*~~*Applicants version is acceptable*~~.~~

Section A6.6.3 Genotoxicity in vitro

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

Results and discussion	Adopt applicant's version ~~or include revised version. If necessary, discuss relev deviations from applicant's view referring to the (sub)heading number~~with following comments. The paper is relevant to the current review and the comet assay is under consideration by a number of regulatory bodies for addition to the standard genotox test battery. Currently without a OECD guideline and because it is not commonly submitted its position in the permethrin evaluation is difficult to discern. However, it is relevant.~~.~~
Conclusion	Other conclusions: *(Adopt applicant's version or include revised version)*
Reliability	~~*Based on the assessment of materials and methods include appropriate reliability indicator*~~3
Acceptability	N~~acceptable / n~~ot acceptable ~~(give reasons if necessary, e.g. if a study is considered acceptable despite a poo reliability indicator. Discuss the relevance of deficiencies and indicate if repeat is necessary.)~~The study is non-guideline and non-GLP. In addition the substance is not unambiguously identified (Batch number, purity and isomeric ratio are omitted). Also, the cells studied are not commonly used in regulatory toxicology studies.
Remarks	

Formatted: Font: Not Italic

Formatted: Font: Not Italic

Formatted: Font: Not Italic

Formatted: Font: Not Italic

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	

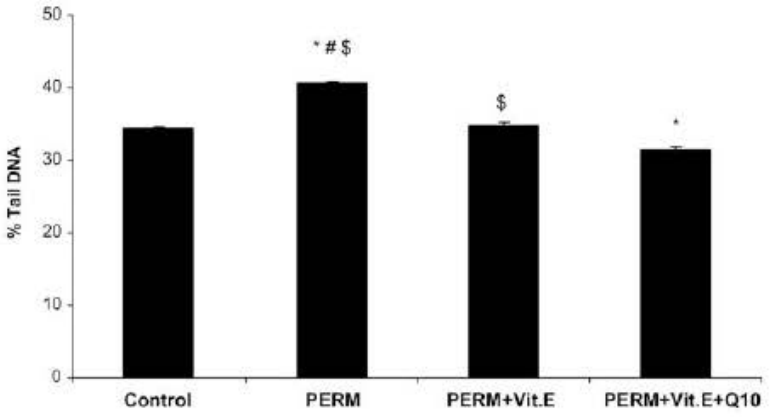

Fig. 1. % Tail DNA in striatum cells after 60 d treatment with permethrin (1/10 LD_{50}) alone or plus antioxidants. The mean values• SEM were obtained after pooling the data from six rats. At least 50 nuclei/slide, for a total of three slides were scored; the same experiment was repeated four times. P• 0.001 * vs. CONTROL, # vs. PERM• Vit.E, $ vs. PERM• Vit.E• Q_{10}.

Section A6.6.4 **Genotoxicity *in vivo***

Annex Point IIA6.6.4 **Mammalian bone marrow chromosome aberration test**

Official use only

55 Reference

55.1	**Reference**	[redacted] (1998) Chromosomal Aberration Study of Permethrin Technical in Mice. Department of Toxicology, [redacted] unpublished report no.: 1594. Dates of experimental work: August 18 – September 28, 1998.
55.2	**Data protection**	Yes
55.2.1	Data owner	Tagros Chemicals India Ltd.
55.2.2	Companies with letter of access	Not applicable
55.2.3	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 [T33]: Confidential

Formatted: Highlight

Formatted: Highlight

56 Guidelines and Quality Assurance

56.1	**Guideline study**	Yes, the test method was based on OECD Guideline 475.
56.2	**GLP**	Yes (certified by the Minister of Health, Welfare and Sport State Supervisory Public Health Service, Veterinary Public Health Inspectorate, the Netherlands)
56.3	**Deviations**	Yes, with the following deviation Dosing was carried out on two consecutive days, as opposed to a single dose as recommended. No scientific justification is given for this dosing regimen This deviation is not considered to compromise the scientific validity of the study.

Section A6.6.4 **Genotoxicity *in vivo***
Annex Point IIA6.6.4 **Mammalian bone marrow chromosome aberration test**

57 MATERIALS AND MethodS

57.1	**Test material**	As given in section 2 (Permethrin 40: 60)
57.1.1	Lot/Batch number	PH 01
57.1.2	Specification	As given in section 2 (Permethrin 40: 60)
57.1.2.1	Description	Light yellow, viscous liquid
57.1.2.2	Purity	92.5%
57.1.2.3	Stability	Not relevant
57.2	**Test Animals**	
57.2.1	Species	Mouse
57.2.2	Strain	Swiss albino
57.2.3	Source	[redacted]
57.2.4	Sex	Male and female
57.2.5	Age/weight at study initiation	5 weeks old on receipt 20 – 30 g
57.2.6	Number of animals per group	5 animals/group/sex
57.2.7	Control animals	Yes
57.3	**Administration**	Oral
57.3.1	Number of applications	2 doses (on 2 consecutive days)
57.3.2	Interval between applications	Not documented
57.3.3	Post exposure period	Not documented
57.3.4	Type	Gavage

Comment [T34]: Confidential
Formatted: Highlight

Section A6.6.4	Genotoxicity *in vivo*	
Annex Point IIA6.6.4	Mammalian bone marrow chromosome aberration test	
57.3.5 Concentration	78.5 (group II), 157 (group III) and 314 (group IV) mg/kg bw	
57.3.6 Vehicle	Peanut oil	
57.3.7 Concentration in vehicle	Permethrin technical was suspended in peanut oil at the required concentrations	X
57.3.8 Total volume applied	10 ml/kg	
57.3.9 Controls	Control group (group I): Peanut oil only (by gavage) Positive control (group V): Mitomycin C (4 mg/kg bw by ip injection)	
57.4 Examinations		
57.4.1 Clinical signs	On day 1 and 2 and before sacrifice for clinical signs of toxicity and mortality	
57.4.2 Tissue	Bone marrow	
	Number of animals:	All animals (5 groups of 10 mice)
	Number of cells:	1000 cells per animal
	Time points:	1 day following final dose (Day 3)
	Type of cells:	Erythrocytes in bone marrow
	Cell preparations:	Bone marrow samples were centrifuged, the supernatant was discarded and 0.075M potassium chloride was added to the cell pellet. This was then mixed well and incubated at 37°C for 30 minutes and then recentrifuged. The supernatant was discarded and freshly chilled Carnoy's fixative (methanol and glacial acetic acid at a 3:1 ratio) was added to the cell pellet to fix the cells. These were maintained at 4°C for a minimum of 12 hours, after which time they were recentrifuged and resuspended in Carnoy's fixative. The tubes were centrifuged again and the supernatant was discarded leaving 0.5 ml of fixative with cell pellet. Two slides for each animal were prepared by dropping 0.5 ml of the fixed cell suspension onto slides. These were dried and stained with 5% Giemsa in phosphate buffer. One slide was used for scoring and one was maintained as a reserve.

Section A6.6.4 **Genotoxicity *in vivo***

Annex Point IIA6.6.4 **Mammalian bone marrow chromosome aberration test**

	Parameters:	A minimum of 100 well spread metaphases per animal were scored under 10 X oil immersion objective. Numbers and types of structural aberrations: Structural (gaps, breaks and fragments) and numerical (ploidy) anomalies and pulverisation were recorded. The number of aberrant cells with one or more aberrations excluding gaps were recorded to calculate percentage aberrant cells.

57.5 Further remarks

Bodyweights were recorded on day 1 and 2 before dosing and also prior to colchicine administration

Mice were sacrificed by cervical dislocation, both femora were excised and the epicondyle tips were removed. Bone marrow was expelled, directly into centrifuge tubes, by flushing with 5 ml PBS.

On the day following the last treatment (Day 3), three hours prior to sacrifice, all animals received an ip injection of an aqueous solution of clochicine at a dose level of 4 mg/kg bw, in order to arrest cell division at metaphase.

Data on body weight, mitotic index and percentage aberrant cells were analysed by Student's t-test (Gad and Weil, 1994)

58 Results and Discussion

58.1 Clinical signs

Day 1:
Symptoms of polyurea in two males at 157 mg/kg bw/day
Symptoms of polyurea and tremors in two male at 314 mg/kg bw/day
Tremors in two female with 314 mg/kg bw/day

Day 2:
Symptoms of polyurea in two mice at 157 mg/kg bw
Symptoms of polyurea (3 mice) and tremors (7 mice) at 314 mg/kg bw

58.2 Chromosomal aberrations to change to tissues

Structural aberrations observed were mainly chromatid breaks resulting in fragments. The numerical anomalies recorded were ploidy. However the percent with aberration cells in mice treated with Permethrin technical at a dose level of 314 mg/kg bw (highest dose) did not differ significantly from that of the control animals.

58.3 Positive control

The mean percentage number of aberrant cells was 7.8 in males and 11.2 in female mice treated with Mitomycin- C.

Section A6.6.4	Genotoxicity *in vivo*
Annex Point IIA6.6.4	**Mammalian bone marrow chromosome aberration test**

58.4	**Genotoxicity**	No chromosomal aberrations in the bone marrow cells of mice treated up to a dose level of 314 mg/kg bw for two consecutive days.
58.5	**Other**	No changes in body weights

59 Applicant's Summary and conclusion

59.1 Materials and methods

Permethrin technical was orally administered to groups of 5 animals/sex/group at the following concentrations: 78.5, 157 and 314 mg/kg bw/day. The positive control group received a single intraperitoneal injection of Mitomycin-C.

This study was conducted according to OECD guideline 475 and is described under point 3 with the following deviation:

> Dosing was carried out on two consecutive days, as opposed to a single dose as recommended. No scientific justification is given for this dosing regimen.

This deviation is not thought to affect the scientific validity of the study.

59.2 Results and discussion

Day 1

Two male mice treated with 157 mg/kg bw exhibited symptoms of polyurea. Two male mice treated with 314 mg/kg bw exhibited symptoms of polyurea and tremors. Two female mice treated with 314 mg/kg exhibited tremors.

Day 2

The same two mice treated with 157 mg/kg bw exhibited symptoms of polyurea. Both male and female mice treated with 314 mg/kg bw displayed symptoms of polyurea (3 mice) and tremors (7 mice) .

All animals appeared normal prior to sacrifice.

There was no effect on body weight in any animal at any dose level.

Structural aberrations observed were mainly chromatid breaks resulting in fragments. The numerical anomalies recorded were ploidy. However the percent with aberration cells in mice treated with Permethrin technical at a dose level of 314 mg/kg bw (highest dose) did not differ significantly from that of the control animals. Results are summarised in Table A6.6.4-1.

Results of the positive control group showed the following: chromatid and chromosomal breaks, fragments, a higher incidence of numerical aberrations and pulverization. The mean percentage number of aberrant cells was 7.8 in males and 11.2 in female mice treated with Mitomycin-C.

Section A6.6.4	Genotoxicity *in vivo*
Annex Point IIA6.6.4	**Mammalian bone marrow chromosome aberration test**

59.3 Conclusion	Under the experimental conditions of this study, Permethrin technical did not induce chromosomal aberrations in the bone marrow cells of mice treated up to a dose level of 314 mg/kg bw for two consecutive days.
59.3.1 Reliability	1
59.3.2 Deficiencies	One deviation was noted and is 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	17th June 2009
Materials and Methods	The applicants version is acceptable. An explanation for the dosing regime would be interesting to indicate why the test substance was provided on two consecutive days. It is accepted this dosing regime did not compromise the scientific validity of the study.
Results and discussion	The applicant's version is adopted. The dosing is acceptable with the effects noted indicative of the effects due to Permethrin and is used as an indicator that the test substance reached the bone marrow.
Conclusion	Under the experimental conditions of this study, Permethrin technical did not induce chromosomal aberrations in the bone marrow cells of mice treated up to a dose level of 314 mg/kg bw for two consecutive days
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*

Section A6.6.4 Genotoxicity *in vivo*

Annex Point IIA6.6.4 **Mammalian bone marrow chromosome aberration test**

Remarks

Table A6.6.4-1: Summary of Chromosomal Aberration in Bone Marrow Cells

Group	Dose	Male		Female	
		Mitotic index	% Aberrant cells	Mitotic index	% Aberrant cells
I	Control	2.122 (0.535)	0.800 (0.837)	2.128 (0.274)	0.800 (0.837)
II	78.5 mg Permethrin technical/kg bw	2.454 (0.485)	1.200 (1.304)	2.204 (0.388)	0.400 (0.894)
III	157 mg Permethrin technical/kg bw	2.091 (0.262)	0.800 (1.095)	2.407 (0.472)	0.600 (0.894)
IV	314 mg Permethrin technical/kg bw	2.564 (0.235)	1.000 (1.000)	2.120 (0.559)	0.600 (0.894)
V	4 mg Mitomycin-C/kg bw (Positive control)	1.847 (0.244)	7.800* (2.049)	2.605 (0.631)	11.200* (2.387)

Values are mean and standard deviation

* = Significant at 5% level ($p \leq 0.05$)

Section A6.6.5 **Annex Point IIA6.6.5**	**Study to examine whether mutagenicity of evidence of DNA damage can be demonstrated in tissue other than bone marrow**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	All the presented *in vitro* genotoxicity tests (Doc IIIA, 6.6.1, Doc IIIA, 6.6.2 and Doc IIIA, 6.6.3) and the *in vivo* test (Doc IIIA, 6.6.4) proved negative. Therefore it is proposed that an *in vivo* test in tissue other than bone marrow is not required.	
Undertaking of intended data submission [ ]	Not applicable	
	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	
Evaluation of applicant's justification	The applicant's justification is accepted	
Conclusion	The applicant's justification is acceptable.	
Remarks	None	
	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 A6.6.6 **Annex Point IIA 6.6.6**	**Test to assess possible germ cell effects**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	All the presented *in vitro* genotoxicity tests (Doc IIIA, 6.6.1, Doc IIIA, 6.6.2 and Doc IIIA, 6.6.3) and the *in vivo* test (Doc IIIA, 6.6.4) proved negative. Therefore it is proposed that an *in vivo* test in germ cell is not required.	
Undertaking of intended data submission [ ]	Not applicable	
	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	
Evaluation of applicant's justification	The applicant's justification is applicable.	
Conclusion	The applicant's justification is acceptable.	
Remarks	None	
	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 A6.6.7 **Annex Point IIA 6.6.7**	**Further testing if metabolites of concern are formed in mammals**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	All the presented *in vitro* genotoxicity tests (Doc IIIA, 6.6.1, Doc IIIA, 6.6.2 and Doc IIIA, 6.6.3) and the *in vivo* test (Doc IIIA, 6.6.4) proved negative. Therefore it is proposed that *in vivo* tests are not required.	
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	16th June 2009	
Evaluation of applicant's justification	The applicant's justification is applicable.	
Conclusion	The applicant's justification is acceptable.	
Remarks	None	
	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 A6.7 **Carcinogenicity**
Annex Point IIA6.7 **Carcinogenicity - Oral administration (2 years, rat)**

60 Reference

Official use only

60.1	**Reference**	[redacted] (2007), Combined chronic toxicity/carcinogenicity study of permethrin technical in Wistar rats, [redacted] unpublished report No.: 14994 Dates of experimental work: May 30, 2005 - June 26, 2007
60.2	**Data protection**	Yes
60.2.1	Data owner	Tagros Chemicals India Ltd.
60.2.2	Companies with letter of access	Not applicable
60.2.3	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 [T35]: Confidential
Formatted: Highlight
Formatted: Highlight

61 Guidelines and Quality Assurance

61.1	**Guideline study**	Yes, the study was conducted according to OECD Guideline 453.
61.2	**GLP**	Yes
61.3	**Deviations**	None

62 MATERIALS AND MethodS

62.1	**Test material**	Permethrin (cis:trans ratio 25:75)
62.1.1	Lot/Batch number	Batch No. P-34, P-11, P-17, P-26 and P-40
62.1.2	Specification	As given in section 2 (Permethrin 25:75)
62.1.2.1	Description	Pale yellow viscous liquid
62.1.2.2	Purity	94.20%, 92.86%, 92.29%, 93.61% and 93.01%

X

Section A6.7 Carcinogenicity

Annex Point IIA6.7 **Carcinogenicity - Oral administration (2 years, rat)**

62.1.2.3	Stability	At study initiation, the proposed formulations were checked for homogeneity and stability by chemical analysis. Three samples from the right, left and centre portions were drawn from each of the low and high dietary concentrations of the test substance and a chemical analysis was done on days 1, 3, 5 and 7. The test substance was considered homogeneous. The stability of the test substance in the diet was dissipated about 50%, 67% and 75% on day 3, 5 and 7 respectively. Therefore, the formulation was prepared daily.	X
62.2	**Test Animals**		
62.2.1	Species	Rat	
62.2.2	Strain	Wistar	
62.2.3	Source	[redacted]	
62.2.4	Sex	Male and female	
62.2.5	Age/weight at study initiation	Age: 4 – 5 weeks Weight: males: 82.46 – 175.06 g Females: 80.80 – 148.47 g	
62.2.6	Number of animals per group		
62.2.6.1	At interim sacrifice	High dose satellite: 20/sex/group Control satellite: 10/sex/group	
62.2.6.2	At terminal sacrifice	50/sex/group	
62.2.7	Control animals	50/sex/group	
62.3	**Administration/ Exposure**	Oral	
62.3.1	Duration of treatment	106 weeks	
62.3.2	Interim sacrifice(s)	Yes, after 53 weeks	
62.3.3	Final sacrifice	106 weeks	
62.3.4	Frequency of exposure	Daily	
62.3.5	Postexposure period	Not relevant	

Comment [T36]: Confidential

Formatted: Highlight

Section A6.7 Carcinogenicity

Annex Point IIA6.7 **Carcinogenicity - Oral administration (2 years, rat)**

62.3.6	Type	In food	
62.3.7	Concentration	Food consumption per day 0, 1500, 3000 and 6000 ppm, (approximately equivalent to 0, 75, 150 and 300 mg/kg bw/day) *ad libitum*	x
62.3.8	Vehicle	None	
62.3.9	Concentration in vehicle	Not applicable	
62.3.10	Total volume applied	Not applicable	
62.3.11	Controls	Basal diet	
62.4	**Examinations**		
62.4.1	Body weight	Weekly during the first 13 weeks and monthly thereafter	
62.4.2	Food consumption	Weekly during the first 13 weeks and at approximately three month intervals thereafter	
62.4.3	Water consumption	Not applicable	
62.4.4	Clinical signs	Yes, once daily	
62.4.5	Macroscopic investigations	Palpable masses	
62.4.6	Ophthalmoscopic examination	Not applicable	
62.4.7	Haematology	Yes	
		Number of animals: 20 animals/sex/group and all animals in satellite groups	
		Time points: After 3, 6, 12, 18 and 24 months of treatment and after 3, 6 and 12 months in the satellite groups (control and high dose).	
		Parameters: Haemoglobin (Hb), packed cell volume (PCV), total red blood cell count (RBC) total white blood cell count (WBC), platelet count and clotting time.	
		Other: Differential count was performed for animals in the highest dose groups and control groups.	

Section A6.7 Carcinogenicity

Annex Point IIA6.7 **Carcinogenicity - Oral administration (2 years, rat)**

62.4.8	Clinical Chemistry	Yes
	Number of animals:	10 animals/sex/group
	Time points:	After 6, 12, 18 and 24 months of treatment and after 3, 6 and 12 months in the satellite groups (control and high dose).
	Parameters:	Total protein (TOT), albumin (Alb), glucose, blood urea nitrogen (BUN), alkaline phosphatase (ALPH), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma glutamyl transpeptidase (GGPT)
	Other	None
62.4.9	Urinalysis	Yes
	Number of animals:	10 animals/sex/group
	Time points:	At 3 months on pooled samples/sex/group and at 6, 12, 18 and 24 months of treatment on individual samples and at 3, 6 and 12 months in the satellite groups (control and high dose).
	Parameters:	Colour, appearance, specific gravity, pH, sediment, protein, glucose, bilirubin, ketone and blood, white blood cells, red blood cells, epithelial cells, casts and crystals.
	Other	None
62.4.10	Pathology	Yes, complete gross examination in all animals. All grossly visible lesions, tumours or lesions suspected to be tumours were preserved.
62.4.10.1	Organ Weights	Yes
	From:	10 animals/sex/group, at interim sacrifice, at terminal sacrifice
	Organs:	Brain, liver, kidneys, adrenals, gonads (testes or ovaries), heart, thyroid, pituitary and spleen.
62.4.11	Histopathology	Yes

Section A6.7 **Carcinogenicity**

Annex Point IIA6.7 **Carcinogenicity - Oral administration (2 years, rat)**

		From:	High dose groups and control groups
		Organs:	Brain (medulla, cerebellar cortex, cerebral cortex), pituitary, thyroid (including parathyroid), thymus, lungs (including trachea), heart, salivary glands, liver, spleen, kidneys, adrenal, oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, rectum, urinary bladder, lymph nodes, pancreas, gonads, uterus, accessory genital organs, female mammary gland, skin, musculature, peripheral nerve, spinal cord (cervical, thoracic, lumbar), sternum with bone marrow and femur (including joint) and eyes
62.4.12	Other examinations	All growth pathology findings including the mass/growth from control (G1), low (G2), intermediate (G3), high dose (G4), control satellite (g5) and high satellite (G6).	
62.5	**Statistics**	Data from experimental groups was compared with control. Bodyweight, bodyweight gain, feed consumption, haematology and clinical chemistry parameters of rats belonging to the control and experimental groups were tested using analysis of variance (ANOVA) followed by Student's Newman - Keul's test. Prior to applying ANOVA, data was tested for homogeneity using Barlett's test.	
62.6	**Further remarks**	None	
		63 Results and Discussion	x
63.1	**Body weight**	Statistically significant treatment related decreases in bodyweight were observed in a dose related manner in the 3000 ppm, 6000 ppm and 6000 ppm satellite dose groups when compared to the control. Please refer to Table IIIA 6.7-1.	
		Statistically significant treatment related decreases in bodyweight gain were observed in a dose related manner in the 3000 ppm, 6000 ppm and 6000 ppm satellite dose groups when compared to the control. Please refer to Table IIIA 6.7-2.	x
63.2	**Food consumption**	Statistically significant differences in both directions in food consumption were observed when compared to the control. Please refer to Table IIIA 6.7-3.	x
63.3	**Water consumption**	Not applicable	

Section A6.7 Carcinogenicity

Annex Point IIA6.7 **Carcinogenicity - Oral administration (2 years, rat)**

63.4	Clinical signs	Several toxicity signs such as alopecia, nasal irritation, dullness, diarrha, exophthalmos, chromodacryorrhea, respiratory disease, polyuria, general body weakness, piloerection, hyperactivity and catalepsy were observed in all the treated groups of animals. None of these signs displayed a dose related increase in frequency and, they were considered as incidental. Specific treatment related neurological signs such as tremor and paralysis were observed and recorded in 6 animals (5 males and 1 female) in the intermediate dose groups, in 60 animals (42 males and 12 females) in the high dose groups and in 14 animals (11 males and 3 females) in the high dose satellite groups during the study period. No other neurological signs were observed.	X
63.5	Macroscopic investigations	No treatment related gross pathological changes were observed. All gross pathological observations were either agonal, incidental, infectious or routinely observed in the Wistar rats of this age.	
63.6	Ophthalmoscopic examination	Not applicable	
63.7	Haematology	No treatment related changes in haematology were observed.	
63.8	Clinical Chemistry	No treatment related changes in clinical chemistry were observed.	
63.9	Urinalysis	No treatment related changes in urinalysis were observed.	
63.10	Pathology	No treatment related gross pathological changes were observed.	
63.11	Organ Weights	No treatment related organ weight changes were observed.	
63.12	Histopathology	No treatment related neoplastic findings were observed. No treatment related non-neoplastic proliferative findings were observed. No treatment related non-neoplastic non-proliferative findings were observed.	
63.13	Other examinations	Tumours were observed in all the treated and control groups of animals. The incidence of tumour was high in control and low dose when compared with all the other groups.	
63.14	Time to tumours	Not relevant	
63.15	Other	None	

Section A6.7 **Carcinogenicity**

Annex Point IIA6.7 **Carcinogenicity - Oral administration (2 years, rat)**

64 Applicant's Summary and conclusion

64.1 Materials and methods

The chronic oral toxicity/carcinogenicity of Permethrin was investigated by orally dosing three groups of 50 rats/sex once daily for 106 weeks at the concentrations of 0, 1500, 3000 and 6000 ppm (equivalent to 0, 75, 150 and 300 mg/kg bw/day). Two additional groups of 10 animals/sex/group and 20 animals/sex/group were treated at the doses of 0 and 6000 ppm for 53 weeks and were sacrificed at the end of week 53.

The study was conducted according to OECD guideline 453 and is described under point 3 with no deviations.

64.2 Results and discussion

Mortality was reported I all treated groups and control groups. A slightly higher incidence of mortality in the high dose group of males. In the females the incidence of mortality was equivalent to controls. These deaths are not treatment related as there was not consistent cause of death in the treated or control animals. X

Statistically significant treatment related decreases in bodyweight were observed in a dose related manner in the 3000 ppm, 6000 ppm and 6000 ppm satellite dose groups when compared to the control.

Please refer to Table IIIA 6.7-1.

Statistically significant treatment related decreases in bodyweight gain were observed in a dose related manner in the 3000 ppm, 6000 ppm and 6000 ppm satellite dose groups when compared to the control. X

Please refer to Table IIIA 6.7-2.

Statistically significant differences in both directions in food consumption were observed when compared to the control. x

Please refer to Table IIIA 6.7-3.

Several toxicity signs such as alopecia, nasal irritation, dullness, diarrha, exophthalmos, chromodacryorrhea, respiratory disease, polyuria, general body weakness, piloerection, hyperactivity and catalepsy were observed in all the treated groups of animals. None of these signs displayed a dose related increase in frequency and, they were considered as incidental.

Specific treatment related neurological signs such as tremor and paralysis were observed and recorded in 6 animals (5 males and 1 female) in the intermediate dose groups, in 60 animals (42 males and 12 females) in the high dose groups and in 14 animals (11 males and 3 females) in the high dose satellite groups during the study period. No other neurological signs were observed.

No treatment related changes in haematology were observed. Although few parameters of haematology values of treated groups showed statistically significant differences when compared with control groups, these results were within the normal limits. These differences were not dose related, they were not repeated over different sampling times and in the high dose groups were not common between the main and satellite groups.

No treatment related changes in clinical chemistry were observed. Although few parameters of clinical chemistry values of treated groups

Section A6.7 Carcinogenicity

Annex Point IIA6.7 **Carcinogenicity - Oral administration (2 years, rat)**

		showed statistically significant differences when compared with control groups, these results were within the normal limits. These differences were not dose related, they were not repeated over different sampling times and in the high dose groups were not common between the main and satellite groups. No treatment related changes in urinalysis were observed. No treatment related gross pathological changes were observed. No treatment related organ weight changes were observed. Statistically significant reductions in the absolute weight of brain, liver and kidneys were observed in the male rats of the high dose reversal group. However, the relative organ weights of these organs were not statistically significant decreased and the effect was not observed in any other main dose groups. No treatment related neoplastic findings were observed. The most commonly observed neoplasm were pars distalis adenoma of pituitary, fibro adenoma and adenoma of the mammary gland, C-Cell adenoma of thyroid gland, and endometrial stromal polyp of uterus. The incidence of these findings was not related to test substance administration. No treatment related non-neoplastic proliferative findings were observed. The most commonly observed non-neoplastic proliferative lesions were islet cell and ductal cell hyperplasia of pancreas, basophilic and clear cell focus in the liver, bile ductule hyperplasia, cortex and medulla hyperplasia of adrenal, pars distalis and pars intermedia hyperplasia of pituitary, C-Cell hyperplasia of thyroid, sex cord stromal hyperplasia of ovary, endometrial stromal hyperplasia of uterus, hyperplasia of mammary gland and bone marrow. The incidence of these lesions was not related to test substance administration. No treatment related non-neoplastic non-proliferative findings were observed. Tumours were observed in all the treated and control groups of animals. The incidence of tumour was high in control and low dose when compared with all the other groups.	
64.3	**Conclusion**	Based on the results of the study, it is concluded that the NOAEL of Permethrin in Wistar rats exposed over a period of 2 years is 1500 ppm (equivalent to 75 mg/kg bw).	X
64.3.1	Reliability	1	
64.3.2	Deficiencies	No	

Section A6.7 Carcinogenicity

Annex Point IIA6.7 **Carcinogenicity - Oral administration (2 years, rat)**

Evaluation by Competent Authorities
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

Evaluation by Rapporteur Member State

Date June 2009

Materials and Methods Acceptable

Results and discussion

The applicant's version is adopted with slight revision.

4/5.1 The mortality data should have been fully reported under this point, given that there is an apparent increase in mortality in the high dose males. The data are summarised under 5.2 but not documented. The mortality table has been added (RMS) as Table A6.7-1.

The slight increase in mortality in the high dose male group may have be related to treatment, but was not statistically significant.

4.1/5.2 There was a reduction in weight gain from 3000 ppm in males and females, which was statistically significant ($p < 0.05$) at some intervals from week 47. Mean weight gains were also slightly reduced at 1500 ppm, reaching statistical significance in males from week 100 and at week 106 in females.

4.2/5.2 It could not be concluded whether the recorded fluctuations in food consumption were related to treatment or not.

Actual calculated test substance intake should have been reported under this point. The approximate values given under 3.3.7 do not appear to have any relationship to the data reported in Report Table 5 (Mean achieved intake of substance). Here the data are reported as the mean of the weekly achieved intake (g/kg b.w)

Dietary conc. Of test substance(ppm)	Approx. intake values reported under 3.3.7 above) (mg/kg bw/day)	Mean achieved intakes (Report Table 5) (g/kg b.w.) Males	Mean achieved intakes (Report Table 5) (g/kg b.w.) Females
0	0	0	
1500	75	3.215 (321 mg)	3.912 (391 mg)
3000	150	6.267 (627 mg)	8.159 (816 mg)
6000	300	12.512 (1251 mg)	16.746 (1675 mg)

4.4 Text error: …. in 60 animals (42 males and 12 females) in the high dose groups....

4.4 Should read .... in 60 animals (42 males and **18** females) in the high dose groups....

5.3 While some statistically significant reductions in mean body weight gain were also observed at the low dose level, this dose may be considered an NOAEL.

Conclusion Other conclusions:

The applicants version is acceptable with the above modifications.

Reliability 1

Acceptability Acceptable

Section A6.7 Carcinogenicity

Annex Point IIA6.7 **Carcinogenicity - Oral administration (2 years, rat)**

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 A6.7-1 Summary of unscheduled mortality (males and females)

Group/Dose (ppm)	Mg/kg b.w./day	Mortality (males)	Mortality (females)	Total mortality
1. control	-	2	5	7
2. 1500	75	2	2	4
3. 3000	150	2	4	6
4. 6000	300	8	3	11
5. control (satellite)	-	0	0	0
6. 6000 ppm (satellite)	300	0	0	0

Table A6.7-2 Summary of body weights (males and females)

Week	0 ppm weight ± SD		1500 ppm weight ± SD		3000 ppm weight ± SD		6000 ppm weight ± SD		Control Satellite weight ± SD		6000 ppm Satellite weight ± SD	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
1	119.91 ± 17.96	110.90 ± 16.11	121.17 ± 14.31	111.73 ± 11.92	119.50 ± 14.81	110.90 ± 13.59	123.49 ± 17.51	112.47 ± 13.76	127.48 ± 13.36	115.47 ± 18.37	125.48 ± 18.57	114.61 ± 13.61
2	156.51 ± 20.69	141.48 ± 16.42	157.17 ± 18.62	139.81 ± 15.62	158.69 ± 22.60	135.01 ± 15.04	159.03 ± 21.33	136.14 ± 15.88	164.46 ± 12.46	147.13 ± 22.37	159.00 ± 21.44	139.09 ± 13.78
3	196.70 ± 28.91	158.49 ± 15.26	192.23 ± 21.83	156.57 ± 18.17	188.34 ± 23.98	154.42 ± 15.34	187.16 ± 25.45	152.08 ± 14.93	193.36 ± 13.44	171.24 ± 20.58	189.82 ± 21.38	160.04 ± 18.60
4	233.94 ± 29.71	176.05 ± 15.72	227.48 ± 25.78	174.46 ± 17.12	226.70 ± 31.36	174.31 ± 16.27	232.73 ± 30.37	174.03 ± 14.98	241.83 ± 11.62	189.53 ± 22.37	236.04 ± 27.20	178.97 ± 19.86
5	262.36 ± 28.92	196.37 ± 17.16	260.92 ± 25.94	191.07 ± 18.51	251.46 ± 30.88	184.36 ± 18.32	255.49 ± 33.39	186.11 ± 15.54	262.55 ± 10.78	215.49 ± 20.98	263.90 ± 30.15	195.75* ± 23.38
6	298.13 ± 31.15	208.69 ± 18.97	293.96 ± 28.51	205.67 ± 19.88	286.42 ± 34.01	198.87 ± 22.70	289.83 ± 35.51	204.24 ± 17.26	305.85 ± 13.03	231.17 ± 21.91	302.34 ± 29.02	209.24* ± 27.24
7	320.11 ± 32.56	219 ± 21.02	315.42 ± 32.65	217.33 ± 21.08	307.89 ± 37.80	212.05 ± 22.74	321.17 ± 39.02	216.43 ± 17.97	332.95 ± 17.40	242.71 ± 24.57	332.18 ± 29.54	221.59 ± 30.95
8	345.90 ± 32.64	225.60 ± 21.65	334.04 ± 34.60	223.60 ± 22.80	327.15 ± 40.26	218.65 ± 24.58	341.82 ± 40.73	223.80 ± 19.01	354.90 ± 17.46	251.05 ± 26.59	350.25 ± 29.68	228.52 ± 30.59
9	356.86 ± 33.31	231.39 ± 23.86	344.79 ± 35.36	229.44 ± 23.83	341.87 ± 41.84	224.26 ± 25.07	357.13 ± 43.40	227.64 ± 210.14	370.37 ± 17.57	259.30 ± 28.05	364.97 ± 32.17	233.84* ± 31.38
10	377.28 ± 36.65	237.91 ± 24.95	361.56 ± 37.83	232.55 ± 24.50	355.49 ± 43.14	230.31 ± 26.37	374.39 ± 45.63	233.62 ± 20.90	386.85 ± 17.61	266.47 ± 28.18	385.52 ± 32.76	237.85* ± 33.90
11	394.75 ± 37.43	244.83 ± 26.06	377.29 ± 40.74	238.87 ± 24.62	372.70 ± 46.51	235.31 ± 27.36	388.59 ± 49.04	239.77 ± 23.69	404.76 ± 17.56	270.87 ± 30.12	403.80 ± 35.75	242.19* ± 34.12
12	397.30 ± 38.19	248.72 ± 28.08	384.61 ± 41.72	244.34 ± 26.15	378.48 ± 43.40	238.01 ± 28.51	397.65 ± 48.10	241.02 ± 22.37	415.88 ± 22.82	271.85 ± 31.32	408.26 ± 33.95	245.89 ± 36.48
13	400.13 ± 37.46	249.85 ± 28.20	387.34 ± 42.57	246.76 ± 26.15	381.18 ± 46.03	239.59 ± 29.31	399.55 ± 49.08	241.60 ± 22.07	415.35 ± 31.54	274.62 ± 30.87	405.50 ± 33.01	246.26* ± 36.09
17	415.50 ± 37.69	257.65 ± 29.52	420.06 ± 47.75	253.81 ± 30.09	410.04 ± 50.32	247.54 ± 29.03	427.79 ± 48.01	247.98 ± 23.84	444.53 ± 32.21	290.87 ± 36.27	422.30 ± 32.62	252.70* ± 37.09

Week	0 ppm weight ± SD		1500 ppm weight ± SD		3000 ppm weight ± SD		6000 ppm weight ± SD		Control Satellite weight ± SD		6000 ppm Satellite weight ± SD	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
21	433.59 ± 44.65	264.16 ± 28.84	430.52 ± 50.55	260.90 ± 30.59	431.10 ± 53.14	255.16 ± 29.33	438.66 ± 46.28	252.07 ± 24.49	466.45 ± 29.00	298.52 ± 43.03	437.27* ± 36.66	259.34* ± 35.86
27	462.59 ± 45.83	274.42 ± 31.86	454.89 ± 55.86	267.12 ± 31.86	453.53 ± 56.44	263.35 ± 32.87	459.00 ± 45.97	261.02 ± 25.11	475.63 ± 32.35	315.41 ± 51.08	457.60 ± 35.01	267.80* ± 40.06
30	481.69 ± 48.53	278.41 ± 33.94	472.54 ± 57.62	276.32 ± 33.50	465.71 ± 62.08	269.33 ± 36.37	478.85 ± 47.78	268.04 ± 25.04	498.75 ± 32.16	325.60 ± 56.01	476.38 ± 37.87	270.42* ± 41.18
34	506.48 ± 53.88	287.36 ± 37.17	493.67 ± 60.76	276.81 ± 35.95	482.74 ± 63.89	273.09 ± 36.94	496.08 ± 50.79	273.64 ± 27.90	521.19 ± 34.05	335.64 ± 62.85	494.28 ± 34.94	275.72* ± 41.86
39	527.84 ± 58.19	293.40 ± 39.58	511.55 ± 67.51	284.91 ± 40.23	495.83 ± 67.51	274.79 ± 36.62	515.07 ± 54.08	273.74 ± 29.48	539.84 ± 38.10	344.29 ± 73.10	514.08 ± 38.35	276.84* ± 48.86
43	539.89 ± 58.42	300.86 ± 44.14	522.61 ± 83.38	291.48 ± 45.68	504.79 ± 69.70	283.42 ± 40.41	520.77 ± 61.38	277.81* ± 31.67	561.68 ± 44.58	357.87 ± 81.51	511.42 ± 86.54	280.62 ± 52.57
47	544.92 ± 59.77	300.49 ± 43.53	526.39 ± 79.47	292.90 ± 49.23	501.13 ± 72.16	278.90* ± 40.58	518.66 ± 63.23	277.64* ± 33.11	576.89 ± 48.28	370.36 ± 79.80	535.02* ± 43.85	283.63* ± 52.64
51	551.08 ± 60.72	306.04 ± 45.46	527.74 ± 86.15	298.33 ± 54.62	503.11* ± 76.35	282.33 ± 47.80	519.44 ± 63.46	283.28* ± 36.03	579.02 ± 57.07	375.39 ± 81.03	531.31* ± 51.03	294.03* ± 56.76
55	556.23 ± 65.69	304.27 ± 51.48	527.87 ± 100.43	296.33 ± 61.25	505.38* ± 85.90	278.62 ± 49.37	521.97 ± 75.35	279.84* ± 38.51	-	-	-	-
59	556.02 ± 71.86	313.29 ± 54.01	535.59 ± 100.70	294.66 ± 57.17	498.48* ± 83.91	280.12* ± 50.57	517.66 ± 76.32	280.96* ± 37.38	-	-	-	-
66	558.77 ± 82.36	316.61 ± 60.07	546.82 ± 95.14	295.56 ± 61.16	515.03 ± 101.57	289.09* ± 57.11	520.45 ± 87.65	283.94* ± 46.68	-	-	-	-
69	562.39 ± 88.19	306.38 ± 57.92	550.88 ± 91.09	296.19 ± 58.87	508.77* ± 103.90	287.31 ± 54.87	508.06* ± 95.74	284.24* ± 46.31	-	-	-	-
74	563.56 ± 98.27	314.48 ± 63.30	553.96 ± 93.47	309.00 ± 67.02	508.62* ± 115.92	296.12 ± 59.73	514.89* ± 99.80	303.64 ± 56.30	-	-	-	-
79	544.37 ± 111.66	320.79 ± 62.56	541.98 ± 107.42	309.97 ± 66.15	499.80 ± 115.92	297.50 ± 95.51	494.76 ± 106.71	305.32 ± 62.03	-	-	-	-
83	533.20 ± 110.56	327.09 ± 66.98	517.98 ± 108.38	309.62 ± 70.64	486.70 ± 103.90	306.42 ± 61.83	494.74 ± 107.95	317.28 ± 65.57	-	-	-	-

Week	0 ppm weight ± SD		1500 ppm weight ± SD		3000 ppm weight ± SD		6000 ppm weight ± SD		Control Satellite weight ± SD		6000 ppm Satellite weight ± SD	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
87	531.26 ± 112.70	331.28 ± 66.24	508.33 ± 114.80	309.87 ± 69.10	495.55 ± 101.91	305.41 ± 64.96	498.17 ± 104.35	308.43 ± 51.02	-	-	-	-
91	540.81 ± 102.74	331.50 ± 72.99	509.22 ± 106.66	305.43 ± 68.41	496.50 ± 102.87	305.35 ± 64.74	498.00 ± 104.41	303.71 ± 59.07	-	-	-	-
95	539.29 ± 98.64	328.89 ± 68.96	511.95 ± 109.79	299.31 ± 58.69	491.83 ± 101.87	298.40 ± 61.79	486.44 ± 109.95	300.54* ± 63.41	-	-	-	-
100	582.18 ± 79.15	349.37 ± 69.34	538.14* ± 102.27	310.02 ± 55.26	506.00 ± 98.87	306.40* ± 60.77	504.77* ± 104.48	310.95* ± 61.55	-	-	-	-
103	589.70 ± 75.78	344.03 ± 61.73	543.79* ± 99.28	318.80 ± 53.77	521.05 ± 98.76	312.13 ± 70.01	529.09* ± 91.82	315.36 ± 63.84	-	-	-	-
106	604.46 ± 75.01	357.87 ± 65.33	559.39* ± 101.06	330.41* ± 45.84	528.16 ± 101.98	313.41* ± 70.26	526.01* ± 92.29	303.09* ± 63.50	-	-	-	-

* significantly different from control, $p < 0.05$

Table A6.7-3 Summary of body weight gains (males and females)

Week	0 ppm weight ± SD		1500 ppm weight ± SD		3000 ppm weight ± SD		6000 ppm weight ± SD		Control Satellite weight ± SD		6000 ppm Satellite weight ± SD	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
1	0	0	0	0	0	0	0	0	0	0	0	0
2	36.60 ± 12.37	30.58 ± 8.25	35.99 ± 9.83	28.09 ± 9.24	39.20 ± 12.42	24.11* ± 5.83	35.54 ± 7.76	23.66* ± 7.47	36.99 ± 1.14	31.66 ± 6.48	33.52 ± 6.68	24.47* ± 5.19
3	76.80 ± 18.06	47.59 ± 9.81	71.05 ± 14.10	44.84 ± 13.40	68.84* ± 15.90	43.52 ± 12.73	63.67* ± 14.83	39.61* ± 7.56	65.88 ± 5.85	55.77 ± 10.45	64.34 ± 9.15	45.43* ± 10.00
4	114.03 ± 18.03	65.15 ± 11.48	106.31 ± 17.81	62.74 ± 14.03	107.02 ± 22.63	63.41 ± 15.19	109.24 ± 19.05	61.56 ± 10.19	114.35 ± 8.97	74.06 ± 22.30	110.56 ± 16.37	64.36 ± 13.59
5	142.46 ± 20.10	85.47 ± 14.93	139.75 ± 21.15	79.34* ± 15.46	131.96 ± 23.12	73.46* ± 16.74	132.00* ± 22.44	73.63* ± 12.03	135.07 ± 8.49	100.02 ± 17.69	138.41 ± 18.33	81.13* ± 17.16
6	178.23 ± 24.80	97.79 ± 16.01	172.79 ± 24.34	93.94 ± 17.23	166.93 ± 26.27	87.98* ± 21.45	166.34 ± 25.10	91.76 ± 14.93	178.37 ± 8.43	155.70 ± 17.84	176.86 ± 19.02	94.86* ± 21.59
7	200.20 ± 23.06	108.79 ± 18.46	194.25 ± 28.89	105.60 ± 18.81	188.40 ± 30.85	101.15 ± 21.91	197.68 ± 29.12	103.96 ± 16.54	205.47 ± 12.28	127.24 ± 20.28	206.69 ± 19.74	106.98* ± 25.37
8	225.99 ± 23.56	114.70 ± 20.06	212.87 ± 31.02	111.87 ± 20.82	207.65* ± 33.88	107.76 ± 23.79	218.33 ± 30.95	111.33 ± 18.11	227.42 ± 12.46	135.58 ± 22.48	224.76 ± 21.61	113.91* ± 25.76
9	236.95 ± 25.11	120.50 ± 21.48	223.61 ± 32.37	117.72 ± 21.78	222.37 ± 35.65	113.36 ± 24.18	233.64 ± 34.34	115.17 ± 18.41	242.90 ± 12.81	143.83 ± 25.53	239.49 ± 24.87	119.23* ± 27.37
10	257.38 ± 28.83	127.01 ± 22.78	240.39* ± 34.89	120.82 ± 22.50	235.99* ± 37.32	119.42 ± 25.07	250.90 ± 36.77	121.15 ± 19.72	259.37 ± 13.93	151.00 ± 25.00	260.04 ± 26.45	123.23* ± 29.69
11	274.84 ± 29.41	133.93 ± 23.79	256.12* ± 38.02	127.15 ± 22.46	253.21* ± 41.07	124.41 ± 26.44	265.10 ± 40.44	127.30 ± 24.71	277.29 ± 13.66	155.40 ± 26.38	278.32 ± 30.55	127.57* ± 30.65
12	277.39 ± 30.03	137.82 ± 25.83	263.44 ± 39.85	132.61 ± 24.52	258.99 ± 39.97	127.11 ± 28.05	274.16 ± 39.99	128.55 ± 22.77	288.41 ± 18.85	156.38 ± 29.17	282.78 ± 29.56	131.28 ± 33.37
13	280.23 ± 30.03	138.95 ± 25.61	266.16 ± 41.13	135.03 ± 24.67	261.68 ± 41.85	128.69 ± 28.22	276.06 ± 41.61	129.13 ± 22.40	287.88 ± 26.49	159.16 ± 29.03	280.02 ± 27.68	131.65* ± 33.10
17	295.59 ± 35.76	146.76 ± 28.60	298.89 ± 48.96	142.08 ± 28.23	290.54 ± 46.28	136.64 ± 28.24	304.30 ± 42.06	135.51 ± 23.89	317.05 ± 28.85	175.40 ± 37.65	296.81 ± 28.36	138.09* ± 34.62

Week	0 ppm weight ± SD		1500 ppm weight ± SD		3000 ppm weight ± SD		6000 ppm weight ± SD		Control Satellite weight ± SD		6000 ppm Satellite weight ± SD	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
21	313.69 ± 47.68	153.26 ± 28.48	309.35 ± 51.87	149.17 ± 29.14	311.60 ± 48.92	144.26 ± 28.01	315.17 ± 42.21	139.60 ± 24.84	338.97 ± 26.92	183.05 ± 42.26	311.79* ± 34.58	114.73* ± 33.62
27	342.69 ± 46.91	163.52 ± 29.97	333.72 ± 57.76	155.39 ± 31.22	334.04 ± 51.04	152.45 ± 31.36	335.10 ± 42.36	148.55 ± 24.34	348.15 ± 29.84	199.94 ± 48.88	332.11 ± 32.23	153.18* ± 38.15
30	361.78 ± 47.93	167.51 ± 33.07	351.36 ± 59.04	162.59 ± 32.46	346.21 ± 56.40	158.44 ± 34.20	354.95 ± 43.52	155.56 ± 24.70	371.27 ± 29.28	210.14 ± 53.61	350.90 ± 36.30	155.81* ± 39.90
34	386.57 ± 53.16	176.46 ± 34.93	372.49 ± 61.76	165.09 ± 34.82	363.24 ± 58.35	162.19 ± 35.76	372.19 ± 45.23	161.17 ± 27.20	393.72 ± 30.74	220.18 ± 61.31	368.79 ± 33.24	161.11* ± 40.80
39	407.93 ± 57.62	182.50 ± 37.10	390.38 ± 68.05	173.18 ± 39.33	376.33 ± 62.95	163.89 ± 36.10	391.18 ± 48.17	161.27 ± 28.14	412.36 ± 34.53	228.82 ± 70.52	388.59 ± 37.37	162.23* ± 46.83
43	419.99 ± 58.72	189.96 ± 41.31	401.43 ± 83.87	179.75 ± 44.86	385.29* ± 63.21	172.74 ± 40.10	396.87 ± 54.45	165.34* ± 30.42	434.20 ± 39.97	242.40 ± 78.30	385.94 ± 86.72	166.00 ± 49.29
47	425.02 ± 60.11	189.59 ± 40.79	405.21 ± 80.24	181.18 ± 48.33	381.64* ± 65.60	168.22* ± 40.83	394.76 ± 55.78	165.10* ± 33.01	449.42 ± 43.15	254.89 ± 75.34	409.54* ± 54.53	169.02 ± 49.95
51	431.17 ± 62.23	195.14 ± 42.59	406.57 ± 86.34	186.61 ± 53.65	383.62* ± 70.74	171.65* ± 48.01	395.54 ± 56.72	170.74* ± 35.73	451.55 ± 53.48	259.92 ± 76.49	405.83* ± 51.77	179.41 ± 55.61
55	436.32 ± 68.32	193.38 ± 49.18	406.70 ± 100.83	184.60 ± 59.95	385.88* ± 79.52	167.94* ± 50.00	398.07 ± 68.69	167.42* ± 36.90	-	-	-	-
59	436.32 ± 74.32	202.39 ± 51.82	414.44 ± 101.95	182.93 ± 55.90	378.98* ± 77.62	169.43* ± 50.41	393.74 ± 70.11	168.53* ± 36.77	-	-	-	-
66	439.07 ± 87.23	205.71 ± 58.20	425.67 ± 97.85	183.83* ± 57.73	395.53 ± 96.99	178.40* ± 57.52	396.52 ± 81.77	171.52* ± 43.44	-	-	-	-
69	442.70 ± 92.61	195.48 ± 55.49	429.74 ± 96.71	184.46 ± 56.63	389.27* ± 98.74	176.63* ± 54.99	384.14* ± 89.42	171.40 ± 43.16	-	-	-	-
74	443.57 ± 102.45	203.58 ± 59.97	432.81 ± 95.32	197.27 ± 65.06	389.13* ± 111.38	185.44 ± 59.92	390.96* ± 94.73	190.80 ± 53.87	-	-	-	-
79	424.67 ± 116.82	209.71 ± 60.77	420.34 ± 109.09	198.25 ± 64.29	380.10 ± 112.29	186.81 ± 59.30	370.84 ± 102.86	192.48 ± 60.74	-	-	-	-
83	413.50 ± 112.78	215.47 ± 64.77	396.33 ± 110.33	197.89 ± 70.79	366.93 ± 102.78	195.73 ± 62.47	370.56 ± 104.13	204.66 ± 64.34	-	-	-	-

Week	0 ppm weight ± SD		1500 ppm weight ± SD		3000 ppm weight ± SD		6000 ppm weight ± SD		Control Satellite weight ± SD		6000 ppm Satellite weight ± SD	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
87	411.56 ± 116.31	219.66 ± 64.97	386.68 ± 116.78	198.15 ± 66.12	375.78 ± 101.16	194.73 ± 66.01	373.99 ± 98.85	195.79 ± 52.43	-	-	-	-
91	421.11 ± 107.56	220.44 ± 69.91	387.58 ± 108.37	193.70 ± 67.46	376.91 ± 102.46	194.67 ± 65.52	373.31 ± 98.19	191.07 ± 61.69	-	-	-	-
95	419.59 ± 103.03	217.98 ± 65.02	390.31 ± 110.83	187.58 ± 58.04	372.24 ± 100.55	188.27* ± 63.38	361.75* ± 103.34	187.90 ± 66.17	-	-	-	-
100	462.48 ± 81.74	238.46 ± 64.49	416.49* ± 103.00	198.30* ± 55.20	386.41* ± 98.07	196.27* ± 62.96	380.08* ± 99.59	198.31* ± 64.87	-	-	-	-
103	470.01 ± 76.26	233.22 ± 56.85	422.27* ± 100.41	206.81* ± 54.32	401.46* ± 99.14	201.82 ± 72.00	404.70* ± 90.52	202.72 ± 66.81	-	-	-	-
106	484.76 ± 74.96	247.07 ± 60.81	437.86* ± 102.01	218.34* ± 46.88	408.57* ± 102.43	203.49* ± 71.27	401.76* ± 92.63	190.45* ± 66.88	-	-	-	-

* significantly different from control, $p < 0.05$

Table A6.7-4 Summary of food consumption (males and females)

Week	0 ppm weight ± SD		1500 ppm weight ± SD		3000 ppm weight ± SD		6000 ppm weight ± SD		Control Satellite weight ± SD		6000 ppm Satellite weight ± SD	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
1	220.07 ± 22.20	204.33 ± 20.77	223.42 ± 16.36	228.56 ± 48.81	207.69 ± 17.48	187.60 ± 23.79	193.30* ± 29.79	184.44 ± 31.78	173.78 ± 11.48	185.46 ± 9.82	234.09* ± 14.96	183.70 ± 14.95
2	175.33 ± 27.12	199.60 ± 17.29	177.00 ± 19.17	175.25 ± 19.60	175.91 ± 20.80	191.20 ± 17.82	172.71 ± 29.18	195.32 ± 28.65	188.86 ± 23.94	201.76 ± 1.21	206.59 ± 11.61	198.78 ± 6.73
3	216.93 ± 16.50	197.54 ± 28.99	208.00 ± 10.41	197.31 17.43	190.36* ± 12.92	198.67 ± 20.06	212.73 ± 8.25	184.84 ± 22.66	189.55 ± 21.07	180.24 ± 27.39	211.46 ± 9.74	208.84 ± 10.86
4	200.36 ± 15.45	188.43 ± 16.79	195.11 ± 19.09	198.81 ± 14.81	194.40 ± 27.85	185.97 ± 18.69	191.74 ± 20.88	202.20 ± 14.98	175.66 ± 16.91	215.69 ± 11.88	184.80 ± 30.92	193.22 ± 8.58
5	250.15 ± 7.17	202.89 ± 29.25	241.93* ± 3.07	216.97 ± 27.61	238.41* ± 9.84	225.01 ± 33.42	237.22* ± 5.10	218.20 ± 17.62	241.26 ± 2.40	227.94 ± 24.46	239.378 ± 5.28	209.00 ± 13.54
6	265.52 ± 12.53	219.89 ± 24.15	271.38 ± 14.31	223.67 ± 27.61	236.54* ± 16.20	227.38 ± 28.52	274.36 ± 16.32	233.54 ± 33.82	264.99 ± 33.14	252.31 ± 6.74	259.61 ± 9.18	230.16 ± 21.41
7	244.36 ± 12.20	183.94 ± 20.80	242.26 ± 9.04	191.74 ± 32.46	230.27* ± 8.84	214.01 ± 14.19	212.88* ± 14.88	207.72 ± 31.21	231.48 ± 7.40	232.77 ± 2.05	223.57 ± 13.33	228.90 ± 9.36
8	229.08 ± 27.66	199.58 ± 22.97	209.53 ± 35.73	180.81 ± 27.22	197.60 ± 59.50	214.85 ± 39.16	201.53 ± 37.79	192.16 ± 41.67	198.66 ± 13.10	180.65 ± 29.59	248.53* ± 11.68	237.05 ± 22.02
9	253.57 ± 6.24	242.42 ± 18.68	254.75 ± 10.49	204.23* ± 35.94	251.53 ± 9.11	221.25 ± 19.56	251.38 ± 9.06	230.92 ± 13.24	262.17 ± 2.84	251.93 ± 2.64	258.86 ± 6.82	232.89* ± 5.88
10	254.51 ± 10.52	243.44 ± 14.38	264.89 ± 12.68	203.58* ± 27.08	245.72 ± 25.84	208.29* ± 33.00	253.62 ± 12.56	231.32 ± 29.24	266.93 ± 10.91	239.01 ± 20.23	266.51 ± 15.79	209.54 ± 12.23
11	257.48 ± 7.73	213.47 ± 36.80	252.14 ± 13.98	182.02 ± 29.65	232.53* ± 17.11	199.39 ± 38.65	252.83 ± 9.43	213.85 ± 39.42	260.31 ± 10.65	187.06 ± 0.03	258.64 ± 7.62	210.14 ± 40.70
12	235.75 ± 17.09	227.78 ± 12.08	241.05 ± 18.01	168.83* ± 22.34	201.30* ± 23.65	165.63* ± 37.47	217.10 ± 24.15	175.75* ± 35.19	247.00 ± 12.95	198.61 ± 25.76	201.85 ± 29.88	167.24 ± 54.39
13	226.88 ± 19.23	193.45 ± 44.03	231.50 ± 16.10	156.57* ± 22.51	219.53 ± 24.22	173.78 ± 31.14	246.48 ± 13.52	214.78 ± 30.83	288.56 ± 2.94	194.24 ± 3.56	299.03 ± 36.27	194.68 ± 60.74
27	274.78 ± 10.67	243.32 ± 20.21	253.82 ± 19.27	221.79 ± 18.00	261.12 ± 22.79	228.50 ± 36.52	266.45 ± 22.71	224.16 ± 21.60	285.34 ± 3.28	217.88 ± 6.29	279.71 ± 14.34	233.38 ± 22.14

Week	0 ppm weight ± SD		1500 ppm weight ± SD		3000 ppm weight ± SD		6000 ppm weight ± SD		Control Satellite weight ± SD		6000 ppm Satellite weight ± SD	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
40	279.35 ± 15.58	188.38 ± 13.39	277.27 ± 18.92	182.34 ± 18.90	260.41 ± 25.07	196.76 ± 29.51	253.43 ± 28.13	207.78 ± 37.35	222.63 ± 75.09	214.64 ± 1.61	282.52 ± 18.85	188.96 ± 35.53
53	245.26 ± 8.86	185.24 ± 27.10	243.65 ± 16.71	205.08 ± 26.18	255.53 ± 9.70	189.35 ± 24.46	265.69* ± 26.54	196.54 ± 31.57	243.37 ± 5.64	191.05 ± 9.58	250.40 ± 50.35	211.30 ± 30.31
66	267.42 ± 18.96	205.97 ± 30.81	240.97 ± 36.28	178.60 ± 34.39	252.88 ± 26.67	191.82 ± 33.72	236.09 ± 42.37	178.69 ± 40.50	-	-	-	-
79	235.58 ± 34.67	195.82 ± 55.35	225.76 ± 40.26	185.60 ± 17.99	245.50 ± 28.13	178.01 ± 31.40	223.03 ± 40.01	195.81 ± 42.27	-	-	-	-
90	258.54 ± 27.07	167.27 ± 22.24	231.83* ± 20.16	175.92 ± 27.04	214.59* ± 26.34	160.39 ± 33.33	214.96* ± 19.83	150.48 ± 52.76	-	-	-	-
104	301.18 ± 41.61	246.46 ± 56.98	288.23 ± 47.31	255.40 ± 27.68	275.96 ± 29.56	284.65 ± 19.15	302.53 ± 28.58	284.10* ± 9.61	-	-	-	-

* significantly different from control, $p < 0.05$

Section A6.8.1 **Teratogenicity Study**

Annex Point IIA6.8.1 **Oral teratogenicity test in rabbits**

Official use only

65 Reference

65.1	**Reference**	[redacted] (2006a), Teratogenic evaluation of Permethrin Technical in New Zealand white rabbits, [redacted] unpublished report no.: 14997.
		Dates of experimental work: June 15, 2005 – April 12, 2006
65.2	**Data protection**	Yes
65.2.1	Data owner	Tagros Chemicals India Ltd.
65.2.2	Companies with letter of access	Not applicable
65.2.3	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 [T37]: Confidential
Formatted: Highlight
Formatted: Highlight

66 Guidelines and Quality Assurance

66.1	**Guideline study**	Yes, the test method was based on OECD Guideline 414
66.2	**GLP**	**Yes (certified by Bundesinstitut für Risikobewertung / Federal Institute for Risk Assessment, Germany)**
66.3	**Deviations**	None

67 MATERIALS AND MethodS

67.1	**Test material**	Permethrin technical
67.1.1	Lot/Batch number	P-41 and P-17
67.1.2	Specification	As given in section 2

Section A6.8.1 Teratogenicity Study

Annex Point IIA6.8.1 **Oral teratogenicity test in rabbits**

67.1.2.1	Description	Pale yellow viscous liquid
67.1.2.2	Purity	94.04 and 92.29%
67.1.2.3	Stability	The stability of the test substance in vegetable oil was not analyzed because the dosing solutions were prepared fresh daily just prior to administration.
67.2	**Test animals**	
67.2.1	Species	Rabbit
67.2.2	Strain	New Zealand White
67.2.3	Source	[redacted]
67.2.4	Sex	Female
67.2.5	Age/weight at study initiation	Approximately 20-30 weeks old 1898.5 – 3297.1 g
67.2.6	Number of animals per group	Four groups of 20-26 pregnant animals/group
67.2.7	Control animals	Yes, 20 pregnant animals
67.2.8	Mating period	Hand mating procedure. The date of mating was recorded.
67.3	**Administration / Exposure**	Oral
67.3.1	Duration of exposure	Daily from Day 6 (post-mating) to Day 28 of gestation period.
67.3.2	Postexposure period	Not applicable
		Oral
67.3.3	Type	By gavage
67.3.4	Concentration	0 (vehicle control), 125, 250 and 500 mg/kg bw/day
67.3.5	Vehicle	Vegetable oil

Comment [T38]: Confidential

Formatted: Highlight

Section A6.8.1 **Teratogenicity Study**
Annex Point IIA6.8.1 **Oral teratogenicity test in rabbits**

67.3.6	Concentration in vehicle	Not documented
67.3.7	Total volume applied	2.5 ml/kg bw
67.3.8	Controls	Vegetable oil

67.4 Examinations

67.4.1	Body weight	Yes, on Day 0 and every three days thereafter
67.4.2	Food consumption	Yes, daily
67.4.3	Clinical signs	Yes, daily for clinical signs of toxicity from the initiation of dosing to the termination of the study.
67.4.4	Examination of uterine content	Pregnancy status, the number of corpora lutea in each ovary, early and late embryonic deaths in each horn of uterus, viability of foetuses and their sex were observed. The weights of gravid uterus with cervix of the dams were also taken. The pre- and post-implantation loss was calculated.
67.4.5	Examination of foetuses	
67.4.5.1	General	The weight of foetuses was taken prior to euthanasia. All foetuses were examined for external malformations. Heads of approximately half of each litter were examined to detect possible abnormalities.
67.4.5.2	Skeletal examination	Yes
67.4.5.3	Soft tissue	Yes

67.5 Further remarks

Body weight, body weight gain, feed consumption and pathology data (weight of reproductive organs, body weight of foetuses, pre and post implantation loss in dams, corpora lutea count, implantation and skeletal abnormalities in foetuses) were checked for normality. Normal data were subjected to one-way ANOVA. Non-normal data were subjected to Kruskal-Wallis One-Way ANOVA on Ranks. Students Newman-Keul´s test was employed for post ANOVA comparison.

68 Results and Discussion

Section A6.8.1 Teratogenicity Study

Annex Point IIA6.8.1 **Oral teratogenicity test in rabbits**

68.1	**Maternal toxic effects**	Alopecia was observed in few animals at control, 250 and 500 mg/kg bw/day. Diarrhoea and dullness were observed in few animals at 500 mg/kg bw/day. Three animals at 500 mg/kg bw/day showed abortion during the experimental period. One of these animals died. No treatment related effects on maternal body weights. No treatment related effects on gross pathology, weight of reproductive organs, implantation, corpora lutea and viability of foetuses. A statistically significant increase in the pre and post-implantation loss of foetuses was observed at 500 mg/kg bw/day.
68.2	**Teratogenic/embryo toxic effects**	No treatment related effects on foetus body weights. No treatment related changes on external, skeletal and visceral examinations.
68.3	**Other effects**	No treatment related changes on food consumption.

Section A6.8.1 **Teratogenicity Study**

Annex Point IIA6.8.1 **Oral teratogenicity test in rabbits**

69 Applicant's Summary and conclusion

69.1 Materials and methods

Permethrin technical was administered to four groups of 20-26 pregnant New Zealand white rabbits/group at the following concentrations: 0, 125, 250 and 500 mg/kg bw/day from day 6 to 28 of gestation period.

This study was conducted according to OECD guideline 414 and is described under point 3 with no deviation.

•

Section A6.8.1 Teratogenicity Study

Annex Point IIA6.8.1 **Oral teratogenicity test in rabbits**

69.2 Results and discussion

Alopecia was observed in few animals at control, 250 and 500 mg/kg bw/day. Diarrhoea and dullness were observed in few animals at 500 mg/kg bw/day.

Three animals at 500 mg/kg bw/day showed abortion. One of these animals died. One animal showed abortion at 125 mg/kg bw/day.

No treatment related changes in body weights were observed throughout the study. A statistically significant decrease was observed in body weight gain at 500 mg/kg bw/day when compared with the control group. Results are summarised in Table A6.8.1-1.

Few significant changes in food consumption were observed from week 1 to 2 at 250 and 500 mg/kg bw/day. However, these changes were not considered to be treatment related. Food consumption did not show any significant difference from week 3 till the end of the study. Results are summarised in Table A6.8.1-2.

A statistically significant increase in the pre and post-implantation loss of foetuses was observed at 500 mg/kg bw/day. Results are summarised in Table A6.8.1-3.

No treatment related effects were observed on gross pathology, weight of reproductive organs, implantation, corpora lutea and viability of foetuses.

There were no treatment related external malformations at any dose level. One foetus at 125 mg/kg bw/day showed major malformation exencephaly. This malformation was considered to be spontaneous.

Some visceral abnormalities such as cystic dilatation, folded retina and haemorrhages in the cerebellum were observed in all groups including the control group. Therefore, no treatment related abnormalities were observed.

Treatment related skeletal malformations were not observed in any treated group. Most of the observations recorded were minor abnormalities considered as routine variables.

69.3 Conclusion

69.3.5.3 LO(A)EL maternal toxic effects	500 mg/kg bw/day
69.3.5.3 NO(A)EL maternal toxic effects	250 mg/kg bw/day

Section A6.8.1 Teratogenicity Study

Annex Point IIA6.8.1 **Oral teratogenicity test in rabbits**

69.3.5.3 LO(A)EL embryonic/teratogenic effects	Not applicable
69.3.5.3 NO(A)EL embryonic/teratogenic effects	500 mg/kg bw/day
69.3.5.3 Reliability	1
69.3.5.3 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	May 25, 2009
Materials and Methods	Applicants version is acceptable.
Results and discussion	Adopt applicant's version.
Conclusion	NO(A)EL maternal toxic effects 250 mg/kg bw/day NO(A)EL embryonic/teratogenic effects 500 mg/kg bw/day
Reliability	1
Acceptability	Acceptable
Remarks	Maternal toxicity at the top dose was limited to lack of weight gain, reduced food consumption (both statistically significant) and 1 mortality. Of the 3 abortions seen in the top dose group one was associated with maternal mortality.
	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 A6.8.1-1: Summary of female body weights (g) and body weight gains (%)

Assessment time Days	Control	125 mg/kg bw/day	250 mg/kg bw/day	500 mg/kg bw/day
	Mean ± SD			
0	2221.80 ± 327.63	2329.40 ± 342.96	2352.82 ± 310.63	2348.51 ± 255.37
3	2248.84 ± 374.57	2342.74 ± 346.64	2329.54 ± 343.48	2331.96 ± 227.39
6	2277.81 ± 361.15	2381.87 ± 357.23	2394.11 ± 304.20	2350.08 ± 229.90
9	2348.86 ± 310.71	2483.31 ± 368.07	2493.93 ± 259.66	2418.19 ± 235.38
12	2406.44 ± 379.43	2529.96 ± 339.07	2553.96 ± 293.21	2507.11 ± 225.09
15	2529.59 ± 410.71	2664.50 ± 330.55	2651.28 ± 281.88	2599.83 ± 262.16
18	2541.68 ± 404.08	2693.94 ± 381.23	2701.16 ± 301.57	2686.93 ± 277.27
21	2585.54 ± 357.27	2744.82 ± 360.76	2698.66 ± 310.09	2676.02 ± 274.59
24	2621.50 ± 375.97	2725.89 ± 380.08	2727.85 ± 276.69	2659.09 ± 256.86
27	2666.71 ± 379.94	2760.83 ± 379.58	2762.85 ± 323.33	2680.61 ± 244.96
29	2692.95 ± 381.29	2744.57 ± 418.75	2768.92 ± 320.36	2698.84 ± 242.34
Body weight gain (%)	21.62 ± 9.14	18.81 ± 15.98	18.90 ± 15.11	16.14[a] ± 14.91

[a] Statistically significant differences ($p<0.05$)

Table A6.8.1-2: Summary of feed consumption (g) from Day 1 until Day 14

Assessment time Days	Control	125 mg/kg bw/day	250 mg/kg bw/day	500 mg/kg bw/day
	Mean ± SD			
1	108.20 ± 41.06	98.91 ± 33.01	113.45 ± 35.45	115.62 ± 24.67
2	112.60 ± 31.21	113.09 ± 21.14	116.60 ± 17.92	121.12 ± 27.15
3	111.15 ± 26.24	117.50 ± 20.65	114.90 ± 28.24	114.69 ± 33.27
4	106.00 ± 31.16	111.23 ± 35.08	127.70[a] ± 43.89	129.46[a] ± 40.13
5	112.30 ± 44.71	123.00 ± 52.94	122.75 ± 41.89	132.92[a] ± 33.80
6	131.70 ± 35.78	123.27 ± 31.98	141.35 ± 40.21	137.35 ± 31.64
7	123.00 ± 46.51	134.23 ± 48.82	119.95 ± 57.25	114.77 ± 51.04
8	139.10 ± 40.53	122.59 ± 48.90	121.70 ± 37.07	105.42[a] ± 41.75
9	121.45 ± 48.49	130.27 ± 58.77	120.05 ± 34.12	123.04 ± 53.91
10	140.25 ± 43.03	140.00 ± 47.21	119.20[a] ± 30.11	126.35 ± 47.64
11	145.55 ± 31.15	143.18 ± 38.66	121.15[a] ± 55.85	123.15[a] ± 49.44
12	140.15 ± 41.86	152.91 ± 40.87	122.70 ± 39.27	128.81 ± 49.50
13	148.75 ± 23.81	147.77 ± 40.90	145.45 ± 33.81	130.96[a] ± 47.87
14	141.45 ± 30.35	128.18 ± 36.36	146.65 ± 39.98	129.73 ± 61.32

[a] Statistically significant differences ($p<0.05$)

Table A6.8.1-3: Summary of group mean of pre- and post-implantation loss in dam

	Control	125 mg/kg bw/day	250 mg/kg bw/day	500 mg/kg bw/day
	Mean ± SD			
Pre-implantation loss	5.50 ± 13.35	4.85 ± 12.23	7.88 ± 9.79	13.39[a] ± 22.60
Post-implantation loss	5.63 ± 9.03	2.23 ± 5.73	7.92 ± 13.58	11.44[a] ± 27.64

[a] Statistically significant differences ($p<0.05$)

Section A6.8.2 **Multigeneration Reproduction Toxicity Study**
Annex Point IIA6.8.2 **Two generation study in rats**

70 Reference

Official use only

70.1	**Reference**	[redacted] (2006), Oral two generation reproduction toxicity study with Permethrin technical in Wistar rats, [redacted] unpublished report No.: 05-362-2004. Dates of experimental work: June 13, 2005 – April 15, 2006
70.2	**Data protection**	Yes
70.2.1	Data owner	Tagros Chemicals India Ltd.
70.2.2	Companies with letter of access	Not applicable
70.2.3	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

Comment [T39]: Confidential
Formatted: Highlight
Formatted: Highlight

71 Guidelines and Quality Assurance

71.1	**Guideline study**	Yes, the test method was based on OECD Guideline 416.
71.2	**GLP**	Yes (certified by Bundesinstitut für Risikobewertung / Federal Institute for Risk Assessment, Germany) X
71.3	**Deviations**	None

72 MATERIALS AND MethodS

72.1	**Test material**	As given in section 2 (Permethrin 25:75)
72.1.1	Lot/Batch number	P-34, P-41 and P-17
72.1.2	Specification	As given in section 2

Section A6.8.2 Multigeneration Reproduction Toxicity Study
Annex Point IIA6.8.2 **Two generation study in rats**

72.1.2.1	Description	Pale yellow viscous liquid
72.1.2.2	Purity	94.20, 94.04 and 92.29%, respectively
72.1.2.3	Stability	The stability of the test substance in vegetable oil was not analysed because the dosing solutions were prepared fresh daily just prior to administration.
72.2	**Test Animals**	
72.2.1	Species	Rat
72.2.2	Strain	Wistar
72.2.3	Source	[redacted]
72.2.4	Sex	Male and female
72.2.5	Age/weight at study initiation	8-9 weeks old Males: 209.66 – 312.45 g Females: 142.51 – 220.15 g
72.2.6	Number of animals per group	Four groups of 20 animals/sex/dose
72.2.7	Mating	**After the treatment, males and females in each group were housed in 1:1 ratios (male:female). After ascertaining pregnancy, males were separated and returned to the respective cages and the treatments were continued till sacrifice.**
72.2.8	Duration of mating	Each day, the females were examined for presence of sperm by vaginal smear or vaginal plug. The mating of animals was continued for 2 weeks, if the sperm or vaginal plug was not observed. If the mating was not successful after two weeks, the females were re-mated with proven males of the same group.
72.2.9	Deviations from standard protocol	None
72.2.10	Control animals	Yes (vegetable oil)
72.3	**Administration/ Exposure**	Oral
72.3.1	Animal assignment to dosage groups	Animals were randomised and assigned to four groups on the first day of acclimatisation. Males and females were assigned to groups in such a way as to almost equalise the mean group body weights.

Comment [T40]: Confidential
Formatted: Highlight

X

Section A6.8.2 Multigeneration Reproduction Toxicity Study
Annex Point IIA6.8.2 **Two generation study in rats**

72.3.2	Duration of exposure before mating	85 days	
72.3.3	Duration of exposure in general P, F1, F2 males, females	Over a two generation period. The process of dosing rats with the test substance was continued without interruption, covering the periods of pre-mating, mating, pregnancy, lactation, weaning and during the resting of parental males and females.	
		Oral	
72.3.4	Type	By gavage	
72.3.5	Concentration	0, 125, 250 and 500 mg/kg bw/day	
72.3.6	Vehicle	Vegetable oil	
72.3.7	Concentration in vehicle	Not documented	
72.3.8	Total volume applied	10 ml/kg bw	
72.3.9	Controls	Yes, vegetable oil	
72.4	**Examinations**		
72.4.1	Clinical signs	Yes, daily	
72.4.2	Body weight	Parental animals (P and F_1) were weighed on the first day of dosing and weekly thereafter. Parental females (P and F_1) were weighed on gestation days 0, 7, 14 and 20 or 21 and during lactation on the same days as the weighing of litters and on the day the animals were killed. Live pups (litters) were weighed on the morning after birth and on days 4 and 7 weekly thereafter until termination of the lactating period.	
72.4.3	Food/water consumption	Daily feed consumption was recorded during pre-mating, gestation, lactation and weaning periods.	
72.4.4	Oestrus cycle	Not documented	X

Section A6.8.2 **Multigeneration Reproduction Toxicity Study**
Annex Point IIA6.8.2 **Two generation study in rats**

72.4.5	Sperm parameters	For all P and F_1 males, at termination, testis and epididymis were weighted and one of each organ (right side) preserved for histopathological examination. The left testes and cauda epididymis were used for enumeration of homogenisation-resistant spermatids and cauda epididymal sperm reserves, respectively, in all males of each group of P and F_1 males. P and F_1 males sperm from the cauda epididymis were collected for evaluation of sperm count, motility and morphology. The total number of homogenisation-resistant testicular spermatids was evaluated.
72.4.6	Offspring	Each litter was examined after delivery to establish the number and sex of pups, stillbirths, live births and the presence of gross anomalies. Physical or behavioural abnormalities in the offspring were observed once daily until weaning.
72.4.7	Organ weights P and F1	Brain, liver, spleen, adrenals, kidneys, gonads, thyroids, seminal vesicle with coagulating gland, epididymis, prostate, pituitary, cauda epididymis, thymus (females only) and uterus for P and F_1 animals. Brain, spleen and thymus for F_1 and F_2 pups.
72.4.8	Histopathology P and F1	Organs and tissues of parental (P and F_1) animals were fixed and stored in a suitable medium for histopathological examination: vagina, uterus with cervix, and ovaries, one testis, one epididymis, seminal vesicles, prostate and coagulating gland, previously identified target organs from all P and F_1 animals selected for mating.
72.4.9	Histopathology F1 not selected for mating, F2	Organs of F_1 and F_2 pups were collected and preserved as with parent.
72.5	**Further remarks**	At the time of termination, or death during the study, all parental animals (P and F_1), all pups with external abnormalities or clinical signs, as well as at least one randomly selected pup/sex/litter from both the F_1 and F_2 generation, were examined macroscopically for any structural abnormalities or pathological changes. Pups that were humanely killed in a moribund condition and dead pups, when not macerated, were examined for possible defects and/or cause of death and preserved. The uteri of all primiparous females were examined, in a manner which does not compromise histopathological evaluation, for the presence and number of implantation sites.

73 Results and Discussion

73.1 Effects

Section A6.8.2 Multigeneration Reproduction Toxicity Study
Annex Point IIA6.8.2 **Two generation study in rats**

73.1.1	Parent males	No treatment related clinical signs of toxicity or deaths
		No treatment related changes in body weights and food consumption
		No difference in sperm counts, motility, morphology and spermatid nuclei count
		No test substance related gross pathological findings
		No treatment related changes in organ weights
73.1.2	Parent females	No treatment related clinical signs of toxicity or deaths
		No treatment related changes in body weights and food consumption
		No statistically significant differences in copulation and fertility indices and gestation length
		No test substance related gross pathological findings
		No statistically significant changes in organ weights
73.1.3	F1 males	No treatment related clinical signs of toxicity or deaths
		No treatment related changes in body weights
		No statistically significant differences in litter size, survival indices and sex ratio
		No developmental anomalies, gross and histological lesions were observed
		Statistically significant changes in absolute and relative spleen weights. However, no histopathological changes could be attributed to these changes.
73.1.4	F1 females	No treatment related clinical signs of toxicity or deaths
		No treatment related changes in body weights
		No statistically significant differences in litter size, survival indices and sex ratio
		No developmental anomalies, gross and histological lesions were observed
		No statistically significant changes in organ weights

Section A6.8.2 **Multigeneration Reproduction Toxicity Study**
Annex Point IIA6.8.2 **Two generation study in rats**

73.1.5	F2 males	No treatment related clinical signs of toxicity or deaths No treatment related changes in body weights No statistically significant differences in litter size, survival indices and sex ratio No developmental anomalies, gross and histological lesions were observed Statistically significant changes in relative spleen and thymus weights. However, no histopathological changes could be attributed to these changes.
73.1.6	F2 females	No treatment related clinical signs of toxicity or deaths No treatment related changes in body weights No statistically significant differences in litter size, survival indices and sex ratio No developmental anomalies, gross and histological lesions were observed Statistically significant changes in absolute and relative spleen weights and in relative thymus weight. However, no histopathological changes could be attributed to these changes.
73.2	**Other**	None

74 Applicant's Summary and conclusion

74.1	**Materials and methods**	Permethrin was administered to 4 groups of 20 Wistar rats sex/group at concentrations of 0, 125, 250 and 500 mg/kg bw/day over a two generation period. This study was conducted according to OECD guideline 416 and is described under point 3 with no deviations.
74.2	**Results and discussion**	No treatment related effects were observed on clinical signs of toxicity, body weight and food consumption throughout the two generations. Two P rats, one at 250 mg/kg bw/day and one at 500 mg/kg bw/day died. However, these mortalities were not considered to be treatment related. In the offspring, no adverse effects were observed on the survival and growth in both the F_1 and F_2 animals. No statistically significant changes were observed in mating

performances, sperm parameters, pregnancy, fertility rates, and gestation length in both P and F_1 animals.

The sex ratio and litter size of F_1 and F_2 litters in the treated groups did not differ statistically when compared to the control group.

No treatment related gross and histopathological findings were observed throughout the two generations.

Treatment related organ weight changes were observed in liver of P and F_1 females, uterus of F_1 females, spleen of F_1 males, spleen of F_1 female weanlings, spleen and thymus of F_2 male and female weanlings. However, statistically significant changes were only observed in absolute and relative spleen weights in F_1 males, in relative spleen and thymus weights in F_2 males and in absolute and relative spleen weights and in relative thymus weight in F_2 females. No histopathological changes could be attributed to the change in organ weights. Therefore, the organ weight changes were not considered to be adverse effects. Results are summarised in Table A6.8.2-1 and Table A6.8.2-2.

74.3 Conclusion

Based on the findings observed under the conditions of this study, the dose of 500 mg/kg bw/day was established as the parental and reproductive toxicity NOAEL. No adverse effects were observed in pups up to 500 mg/kg bw/day in both two generations. Therefore, the NOAEL for both parent and pup generations is 500 mg/kg bw/day.

74.3.1 LO(A)EL

74.3.1.1 Parent males — Not applicable

74.3.1.2 Parent females — Not applicable

74.3.1.3 F1 males — Not applicable

74.3.1.4 F1 females — Not applicable

74.3.1.5 F2 males — Not applicable

74.3.1.6 F2 females — Not applicable

74.3.2 NO(A)EL

Section A6.8.2 Multigeneration Reproduction Toxicity Study
Annex Point IIA6.8.2 **Two generation study in rats**

74.3.2.1 Parent males	500 mg/kg bw/day for toxicity and reproductive toxicity
74.3.2.2 Parent females	500 mg/kg bw/day for toxicity and reproductive toxicity
74.3.2.3 F1 males	500 mg/kg bw/day for toxicity and reproductive toxicity
74.3.2.4 F1 females	500 mg/kg bw/day for toxicity and reproductive toxicity
74.3.2.5 F2 males	500 mg/kg bw/day for toxicity
74.3.2.6 F2 females	500 mg/kg bw/day for toxicity
74.3.3 Reliability	1
74.3.4 Deficiencies	None

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	27th May 2009
Materials and Methods	2.2 GLP. The document is unsigned. The statement of GLP compliance, quality assurance assessment and certificate of affirmation are all unsigned in the copy evaluated. 3.2.9 The study did not achieve the desired number of 20 animals pregnant in all dose groups. It may have been wise to start the study with more than 20 animals per dose group. This would have allowed for lack of fertility. 3.4.4 Monitoring of oestrous cycle is required under guidance however this was not carried out.
Results and discussion	Adopt applicant's version
Conclusion	Adopt applicant's version
Reliability	2
Acceptability	Acceptable
Remarks	The apparent lack of toxicity at the high dose is a concern. The dose of 600 mg/kg bw/d elicited Clinical signs in the range-finding study and this dose may have been more appropriate than the top dose selected. The results of the acute oral study yielded a LD_{50} of 554 mg/kg suggesting 500 mg/kg bw/d as an appropriate top dose. However, This study was performed using a different isomeric ratio that may be more toxic than 25:75 ratio used in this study. In summary, the rationale for dose selection appears sound, however, the lack of toxicity at the high dose was not ideal.
	Comments from ...
Date	*Give date of comments submitted*

Section A6.8.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA6.8.2 **Two generation study in rats**

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	