Section A6.4.2 Subchronic dermal toxicity test

Annex Point IIA 6.4

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible** [ ] **Scientifically unjustified** [X]	
Limited exposure [ ]	**Other justification** [X]	
Detailed justification:	The conduct of a percutaneous 90-day toxicity study in the rat is not considered to be required since, in view of the availability of a dermal absorption study and the acute non-toxicity of Alphacypermethrin following topical administration, route-to-route extrapolation is not considered to be restricted in any way. However, a subchronic dermal toxicity study in the rabbit (non-GLP) is available as supportive data.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	March, 2009	
Evaluation of applicant's justification	Skin irritating properties were observed in the rabbit (A6.1.4/01, A6.1.4/02 and slightly irritating in A6.1.1/01). A percutaneous 90-day toxicity study in the rat would have been interesting to be performed.	
Conclusion		
Remarks	none	
	COMMENTS FROM APPLICANT	
Date	07 May 2009	

Evaluation of applicant's justification	We consider the above remark (i) inconclusive and (ii) scientifically questionable, for the following reasons: (i) The RMS' remark does not report on a decision if the justification for non-submission is accepted or not. We courteously request this to be clearly stated. (ii) A positive result in a skin irritation does by no means trigger the conduct of a dermal repeated dose toxicity study. Irritation is a local effect that is appropriately and fully addressed by the standard skin irritation test following EC method B.4. Repeated dose testing via the dermal route would not contribute to existing knowledge and is therefore unnecessary. The TGD on risk assessment (2003), part I, is very clear in this respect (p. 183): "*For skin and eye irritation, it should be very rarely, if ever, necessary to require further testing.*" We further note that animal testing should not be driven by pure scientific inquisitiveness, but exclusively by the regulatory goal to establish safe exposure levels for human health and the environment. The above remark in its current form is suggestive of unnecessary animal testing, thus in contradiction to applicable European law, and should therefore be deleted.
Conclusion	
Remarks	
	COMMENTS FROM RAPPORTEUR MEMBER STATE
Date	May,2009
Conclusion	Remarks of applicant accepted and therefore the justification of non-submission of data is accepted

Section A6.4.2 Subchronic dermal toxicity tests in rabbits

Annex Point IIA6.4 – Supportive data –

The following reference is considered to contain additional information concerning subchronic dermal toxicity of Alphacypermetrin and is thus presented in an abbreviated format (adopted from the PPP-dossier) as supportive data:

The following study was performed on cypermethrin. Therefore, the information given in the report is considered to represent supportive data only. Furthermore, route-to-route extrapolation from the standard repeated-dose toxicity studies via the oral route is not considered to be restricted in any way.

Reference: **A6.4.2/01**

[redacted] (1981): Subacute dermal toxicity study in rabbits with technical cypermethrin. [redacted], Report no. CTL/P/588, February 04, 1981. BASF RDI No.: CY-420-002. (unpublished).

Guidelines: Guideline not stated but, the conduct of the study was consistent to EU method B.9 (92/69/EC) in all important aspects.

GLP: No

Material and methods:

No study of the subacute dermal toxicity of Alphacypermethrin has been conducted. A subacute dermal toxicity of Cypermethrin has been conducted and is summarized here. The considerable similarity between these two compounds with regard to chemical class and general toxicity characteristics allows one to make a reasonable judgement regarding the subacute dermal toxicity of Alphacypermethrin from data gathered for Cypermethrin.

Cypermethrin was applied, at 2, 20 or 200 mg/kg to the skins of groups of ten male and ten female New Zealand white rabbits. The test substance was kept in contact by means of occlusive dressings for six hours per day, five days per week for three weeks (a total of fifteen applications). Fourteen male and 13 female rabbits served as controls and were treated with the vehicle. The skin of one half of the animals in each group was abraded prior to the initial treatment, and once a week thereafter.

Findings:

Repeated application of Cypermethrin caused slight to moderate skin irritation in 8 of 20 rabbits treated at 2 and 18 of 20 rabbits treated at 20 mg/kg bw/d, as assessed on a Draize scale. A slight to severe skin irritation in 19 of 20 rabbits treated at 200 mg/kg bw/d was also observed. Several control animals also exhibited slight to moderate irritation. Clinical signs revealed no treatment-related effects. While most test and control animals lost weight, analysis of body weight showed statistically significant effects only in female (abraded) animals at 200 mg/kg bw/d. Although not statistically significant, males treated at 200 mg/kg bw/d also exhibited increased body weight loss. Analysis of food consumption showed no treatment-related effects. There were no haematological or clinical chemistry changes observed which were considered treatment-related.

Analysis of organ weights showed a significant reduction in absolute mean testis weight in male rabbits administered 200 mg/kg bw/d. Although not statistically significant, the testis-to-body weight ratio for males in this dose group was also decreased. There were no gross treatment-related findings at necropsy.

The only histopathological finding which may have been due to the administration of Cypermethrin was focal liver necrosis in six female and 3 male rabbits given 200 mg/kg b.w./day. Focal liver necrosis was also present in two males given 20 mg/kg bw/d, in 2 males and 1 female given 2 mg/kg bwd, and in 2 male and 2 female control animals. The incidence of focal liver necrosis in the 20 and 2 mg/kg bw/d dose groups was considered comparable to that of controls.

NOAEL: 20 mg/kg bw/d, based on increased body weight loss in males and females, decreased absolute and relative testis weight in males, and increased incidences of focal liver necrosis in females treated with 200 mg/kg bw/d.

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	March, 2009
Materials and Methods	Applicant's version adopted
Results and discussion	Applicant's version adopted
Conclusion	Applicant's version adopted
Reliability	1 (Guideline not stated but, the conduct of the study was consistent to EU method B.9 (92/69/EC) in all important aspects.)
Acceptability	acceptable
Remarks	none
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.4.3 Subchronic inhalation toxicity test

Annex Point IIA 6.4

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible** [ ] **Scientifically unjustified** [X]	
Limited exposure [ ]	**Other justification** [X]	
Detailed justification:	(1) The vapour pressure of Alphacypermethrin (IIA, 3.2) was determined experimentally under GLP to a value below 1 x 10^{-5} hPa (20°C). Therefore, Alphacypermethrin is not a volatile substance. (2) Although exposure by inhalation may occur to some degree during application of the biocidal product, this route is quantitatively of minor importance. Exposure is expected to be predominantly dermal. In view of the availability of an intestinal absorption rate (45%) derived from the toxicokinetic studies, systemic no-effect levels can be determined for the risk assessment when absorption in the respiratory tract is assumed to amount 100%. In conclusion, the performance of a 90-day inhalation toxicity study in the rat is consequently not considered to be required since route-to-route extrapolation is not restricted in any way.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	March, 2009	
Evaluation of applicant's justification	Applicant's version adopted	
Conclusion	Applicant's version adopted	
Remarks	none	
	COMMENTS FROM ...	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A6.5 **Chronic toxicity and carcinogenicity study in rats**
Annex Point IIA 6.5

Official use only

1 REFERENCE

1.1	**Reference**	**A6.5/01:** [redacted] (1978) 2 year feeding study of WL43467 in rats. [redacted], Report no. TLGR.78.189 (unpublished). BASF RDI No.:CY-427-001 **A6.5/02:** [redacted] (1979) Corrigendum and addendum I: 2 year feeding study of WL43467 in rats. [redacted], Report no. TLGR.78.189, February 1979, (unpublished). BASF RDI No.:CY-427-002 **A6.5/03:** [redacted] (1981) Corrigendum and addendum II: 2 year feeding study of WL43467 in rats. [redacted], Report no. TLGR.78.189, February 1981, (unpublished). BASF RDI No.:CY-427-003 **A6.5/04:** [redacted] (1985) Corrigendum and addendum III: 2 year feeding study of WL43467 in rats. [redacted] Report no. TLGR.78.189, February 1985, (unpublished). BASF RDI No.:CY-427-004
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	No
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No The conduct of the study was consistent to EU method B.30 (88/303/EC) in all important aspects: with the following exceptions: less animals were used - 24 males and 24 females were treated instead of 50 animals per sex, the satellite groups contained 6 or 12 animals per sex instead of 20 animals per sex; adrenals and ovaries were not weighed; no urinalysis; no determination of albumin concentration and blood glucose; no gamma glutamyl transpeptidase and no ornithine decarboxylase were measured.

Section A6.5 **Chronic toxicity and carcinogenicity study in rats**
Annex Point IIA 6.5

2.2	**GLP**	No At the time of the study conduct, GLP was not compulsory. However, the study was conducted in accordance with the principles of GLP.
2.3	**Deviations**	Not applicable

3 MATERIALS AND METHODS

3.1	**Test material**	Cypermethrin (WL 43467)	
3.1.1	Lot/Batch number	30	
3.1.2	Specification	Not specified	
3.1.3	Purity	98%	
3.1.4	Description	Not stated	
3.1.5	Stability	No information available	
3.2	**Test animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Wistar, specific pathogens free (SPF)	
3.2.3	Source	Shell Toxicology Laboratory, Tunstall, GB	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	Age: Mean body weight ranges:	5 weeks 139–141 g (male) 118–121 g (females)
3.2.6	Number of animals per group	In total 48 males and 48 females per group (interim sacrifice month 6: 6 males and 6 females; month 12: 6 males and 6 females; month 18: 12 males and 12 females)	
3.2.7	Control animals	In total 96 males and 96 females (interim sacrifice month 6: 12 males and 12 females; month 12: 12 males and 12 females; month 18: 24 males and 24 females)	
3.3	**Administration/ Exposure**		
3.3.1	Duration of treatment	24 months (interim sacrifices: 6, 12 and 18 months)	
3.3.2	Frequency of exposure	Continuously	
3.3.3	Post-exposure period	None	
3.3.4	Type	Dietary	
3.3.5	Concentration	1, 10, 100 and 1000 ppm	
3.3.6	Solvent	100 ml acetone	

Section A6.5 **Chronic toxicity and carcinogenicity study in rats**
Annex Point IIA 6.5

3.3.7	Concentration in diet	Stability and concentrations in the diets were within acceptable limits throughout the study.
3.3.8	Total volume applied	Diet *ad libitum*
3.3.9	Controls	Control diet with vehicle
3.4	**Examinations**	
3.4.1	Observations	Yes (daily)
	Clinical signs	Yes (daily)
	Mortality	Yes (daily)
3.4.2	Body weight	Yes (weekly for the first 13 weeks, at week 15 and thereafter at 4-week intervals)
3.4.3	Food consumption	Yes (weekly for the first 13 weeks, at week 16 and thereafter at 4-week intervals)
3.4.4	Water consumption	No
3.4.5	Haematology	Yes Number of animals: each surviving animal Time points: end of study Parameters: haemoglobin, red blood cell counts, total white blood cells counts, differential white blood cell counts, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, prothrombin time, kaolin-cephalin clotting time.
3.4.6	Clinical chemistry	Yes Number of animals: each surviving animal Time points: end of study Parameters: protein, urea, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, sodium, potassium, chloride.
3.4.7	Urinalysis	No
3.5	**Sacrifice and pathology**	
3.5.1	Organ weights	Yes Organs: liver, kidneys, testes, spleen, brain and heart.

Section A6.5 **Chronic toxicity and carcinogenicity study in rats**
Annex Point IIA 6.5

3.5.2	Gross and histopathology	Yes Macroscopic observations: all animals which died and all surviving animals. Histopathological examination: brain, heart, liver, spleen, kidneys, testes, ovaries, stomach, pancreas, mesenteric lymph nodes, prostate or uterus, thyroid/parathyroid with oesophagus and trachea, thymus, eye and lachrymal glands, lungs, pituitary, adrenals, small intestine, large intestine, salivary glands, urinary bladder, sciatic nerves. Examinations were performed on organs and tissues from control rats, rats fed 100 and 1000 ppm Cypermethrin, rats that died earlier in these groups and any other macroscopic lesion in any tissue.
3.5.3	Other examinations	None
3.5.4	Statistics	Body and organ weights: covariance analysis. Clinical chemistry and haematology: analysis of variance. Statistical significance: Williams' *t*-test or Dunnett's test, as appropriate. Tumour data: actuarial analysis (Peto, R. 1974)
3.6	**Further remarks**	None

4 RESULTS

4.1	**Observations**	
4.1.1	Clinical signs	The general health and behaviour of treated and control rats were similar throughout the study.
4.1.2	Mortality	The survival after 2 years of males and female rats of the control and treated groups was similar. Results are presented in Table A6.5-1.
4.2	**Body weight**	Male and female rats treated with Cypermethrin at 1000 ppm had reduced body weights throughout the study when compared to controls. Reductions were not always statistically significant with no significant differences occurring in female body weights after week 76. Results are presented in Table A6.5-1.
4.3	**Food consumption and compound intake**	During the first 13 weeks, small, sometimes statistically significant, reductions in food consumption were seen in the 1000 ppm group males and females. Thereafter, only minor fluctuations in food intake were observed. Results are presented in Table A6.5-1.
4.4	**Blood analysis**	
4.4.1	Haematology	In various parameters, minor statistically significant fluctuations were seen in the interim and in the two years groups, but all findings were considered to be of no toxicological significance.

Section A6.5 Chronic toxicity and carcinogenicity study in rats

Annex Point IIA 6.5

4.4.2	Clinical chemistry	No treatment-related changes were observed. Plasma alkaline phosphatase values in males, exposed to 10, 100 and 1000 ppm (2 years) were reduced compared to controls. However, no correlation was noted between exposure level and the magnitude of the change over a 100-fold range, and no evidence of any compound-related pathological changes was seen. Thus, the finding was not considered to be of toxicological evidence. Blood urea values were increased in male rats in the 1000 ppm group sacrificed after 2 years and were marginally increased in high dose females after 1 year. This finding was not accompanied by any changes in the incidence or severity of nephrosis during or at the end of the study. It was regarded as difficult to clearly define a relationship between these findings and exposure to Cypermethrin. Results are presented in Table A6.5-1.
4.4.3	Urinalysis	No
4.5	**Sacrifice and pathology**	
4.5.1	Organ weights	No organ weight changes of toxicological significance were seen that could be attributed to treatment with Cypermethrin. There were significant increases in either absolute or relative organ weights at 1000 ppm for testes (males, 18 months), liver (males, 6 months), heart (males, 6 months), kidney (males, 12 and 18 months; females, 6 months). However, no consistent patterns were seen, no effects were found after two years and no correlating histopathological or clinical chemistry changes were observed. Therefore, these findings were considered not relevant.
4.5.2	Gross and histopathology	No significant treatment-related macroscopic or histological changes were observed in rats fed Cypermethrin at dietary concentrations of 1 to 1000 ppm for up to two years. Sciatic nerves appeared similar at necropsy in all treatment groups and histopathological evaluations did not show any consistent, treatment-related evidence of degeneration.
4.6	**Other**	Statistical analysis revealed no evidence of increased risk of treatment-related tumour development following dietary exposure to Cypermethrin for up to two years.

Section A6.5 **Chronic toxicity and carcinogenicity study in rats**
Annex Point IIA 6.5

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The chronic toxicity and carcinogenicity of Cypermethrin (WL 43467) was tested in 24 Wistar rats per sex fed the test substance at dietary concentrations of 1, 10, 100, and 1000 ppm. A control group of 48 animals per sex received untreated diet. Groups of rats were scheduled for necropsy after 6 months (6 per sex treated, 12 per sex control), 12 months (6 per sex treated, 12 per sex control), 18 months (12 per sex treated, 24 per sex control) and 2 years (24 per sex treated, 48 per sex control) of treatment. Although not a guideline study, the method used was consistent to EU method B.33 (88/303/EC) in all important aspects, with the following exceptions: less tested animals were used (24 males and 24 females were treated instead of 50 animals per sex), the satellite groups contained 6 or 12 animals per sex instead of 20 dosed animals per sex; adrenals and ovaries were not weighed; no urinalysis; no determination of albumin concentration and blood glucose; no gamma glutamyl transpeptidase and no ornithine decarboxylase were measured.
5.2	**Results and discussion**	Male and female rats exposed to 1000 ppm Cypermethrin had reduced body weights throughout the study. The trend was consistent but not always statistically significant. No significant differences in survival were seen between treatment and control groups. There were no treatment-related effects on haematology, clinical chemistry and organ weights. Macroscopic and histopathological examination did not indicate any biologically significant lesions in any organ or other effects that were considered to be related to treatment. A wide spectrum of tumours and degenerative and other lesions were seen at post-mortem and microscopically. None appeared to be compound-related. Based on decreased body weights for both sexes the NOAEL was determined to be 100 ppm.
5.3	**Conclusion**	
5.3.1	LO(A)EL	1000 ppm
5.3.2	NO(A)EL (chronic effects)	100 ppm, corresponding to 5 mg/kg bw/d (applying a factor of 0.05 for adult rats (GDCh, BUA Grundsatzpapiere, August 1992) for the conversion from "ppm in feed" to "mg/kg bw/d")
5.3.3	NO(A)EL (oncogenic effects)	1000 ppm, about 50 mg/kg bw/d
5.3.4	Other	
5.3.5	Reliability	2
5.3.6	Deficiencies	No

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

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	April, 2009
Materials and methods	Applicant's version adopted
Results and discussion	Applicant's version adopted
Conclusion	5.3.3. there is no evidence of increased risk of tumor development following dietary inclusion of 1-1000 ppm WL43467 for up to 2 years.
Reliability	2 (The conduct of the study was consistent to EU method B.30 (88/303/EC) in all important aspects: with the following exceptions: less animals were used - 24 males and 24 females were treated instead of 50 animals per sex, the satellite groups contained 6 or 12 animals per sex instead of 20 animals per sex; adrenals and ovaries were not weighed; no urinalysis; no determination of albumin concentration and blood glucose; no gamma glutamyl transpeptidase and no ornithine decarboxylase were measured.)
Acceptability	acceptable
Remarks	none

	COMMENTS FROM ...
Date	
Materials and methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.5- 1: Results of the 2-year feeding study in rats.

Parameter	Control		1 ppm		10 ppm		100 ppm		1000 ppm		Dose-response +/–	
	m	f	m	f	m	f	m	f	m	f	m	f
No of animals examined												
6 months	12	12	6	6	6	6	6	6	6	6		
12 months	12	12	6	6	6	6	6	6	6	6		
18 months	24	24	12	12	12	12	12	12	12	12		
2 years	48	48	24	24	24	24	24	24	24	24		
Survival rate [%]												
2 years	67	42	46	33	54	38	71	42	71	50	–	–
Body weight [g]												
Week 1	184	160	184	160	185	160	184	159	166**	153**	–	–
Week 13	462	287	462	281	468	286	448*	286	435**[1]	264**	–	–
Week 75	569	403	582	402	582	393	565	419	534**	375**	–	–
Week 103a	552	412	560	384	560	413	524	419	515*	398	–	–
Food intake [g/rat]												
Week 1	135	122	134	121	137	122	133	124	106**	103**	–	–
Week 13	141	132	145	123	139	132	138	131	134*[1]	120**	–	–
Week 103a	112	95	97	78	102	83	108	96	105	86	–	–
Clinical chemistry												
Urea (1 year) [mmol/L]	7.0	9.0	6.7	8.8	6.8	8.9	7.5	8.2	7.5	9.7*		+
Urea (2 years) [mmol/L]	7.8	8.7	7.4	6.7	8.1	6.1	11.3	6.9	12.3**[2]	7.0	+	
AP (2 years) [IU]	72	37	63	32	53*	35	59**	36	56**	40	–	

*) significantly greater or less than control mean ($p<0.05$)
**) significantly greater or less than control mean ($p<0.01$)
1) No. of observations = 21
2) No. of observations = 16, excludes one outlying value, male 80
a) measured one week early

Section A6.5 **Carcinogenicity test in mice**
Annex Point IIA 6.5

Official use only

1 REFERENCE

1.1	**Reference**	**A6.5/05:** [redacted] (1996) Alphacypermethrin: Oncogenicity study by dietary administration to CD 1 mice. Report no. 95/SHL010/0596, May 20, 1996 (unpublished). BASF RDI No.: AL-428-002. **A6.5/06:** [redacted] (1999) Alphacypermethrin: Oncogenicity study by dietary administration to CD 1 mice. First amendment to Report no. 95/SHL010/0596, November 4, 1999 (unpublished). BASF RDI No.: AL-428-003.
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	No
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes OECD guideline 451 (1981) The conduct of the study was consistent to EU method B.32 (88/303/EC) in all important aspects.
2.2	**GLP**	Yes
2.3	**Deviations**	None

3 MATERIALS AND METHODS

3.1	**Test material**	Alphacypermethrin
3.1.1	Lot/Batch number	02156
3.1.2	Specification	As given in Section A2
3.1.3	Purity	95.4%
3.1.4	Description	Aggregative off-white powder
3.1.5	Stability	The test substance was stable during the duration of the study.
3.2	**Test animals**	
3.2.1	Species	Mouse

Section A6.5 Carcinogenicity test in mice

Annex Point IIA 6.5

3.2.2	Strain	CD-1	
3.2.3	Source	Charles River Limited, Margate, Kent, England	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	Age:	30–37 days
		Body weight (means):	27.1 g (males) 22.5 g (females)
3.2.6	Number of animals per group	72 male and 72 female animals (interim phase: 20 animals per sex; terminal phase: 52 animals per sex)	
3.2.7	Control animals	72 males and 72 females (interim phase: 20 animals per sex; terminal phase: 52 animals per sex)	
3.3	**Administration/ Exposure**		
3.3.1	Duration of treatment	78 weeks (interim phase: 52 weeks)	
3.3.2	Frequency of exposure	Continuously	
3.3.3	Post-exposure period	None	
3.3.4	Type	Dietary	
3.3.5	Concentration	30, 100 and 300 ppm.	
3.3.6	Vehicle	None	
3.3.7	Concentration in vehicle	Not applicable	
3.3.8	Concentration in diet	Stability in the diet was confirmed prior to study initiation. Diet sample analysis showed acceptable concentrations (94.7 ± 2.6, 95.3 ± 3.0 and 95.9 ± 3.4 % for 30, 100 and 300 ppm, respectively) and homogeneity.	
3.3.9	Total volume applied	Diet *ad libitum*	
3.3.10	Controls	Control diet	
3.4	**Examinations**		
3.4.1	Observations	Yes (at least twice daily; more detailed examination weekly)	
	Clinical signs	Yes (at least twice daily; more detailed examination weekly)	
	Mortality	Yes (at least twice daily)	
3.4.2	Body weight	Yes (before treatment, weekly for the first 14 weeks of treatment and once every two weeks thereafter and before necropsy)	
3.4.3	Food consumption	Yes (mean weekly consumption was recorded for each cage)	
3.4.4	Food conversion efficiency	Yes (weekly food conversion efficiency was calculated for the first 14 weeks of treatment)	
3.4.5	Water consumption	Yes, (daily by visual appraisal, no quantitative measurements)	

Section A6.5 Carcinogenicity test in mice

Annex Point IIA 6.5

3.4.6	Ophthalmoscopic examination	No
3.4.7	Haematology	Yes Number of animals: each surviving animal of the high dose and control groups and animals sacrificed prematurely Time points: after week 50 and 77 Parameters: differential white blood cell counts
3.5	**Sacrifice and pathology**	
3.5.1	Organ weights	Yes Organs: adrenals, brain, heart, kidneys, liver, lungs with main stem bronchi, spleen, testes, uterus with cervix
3.5.2	Gross and histopathology	Yes Macroscopic observations: Animals of all dose groups were examined by detailed inspection of external features and orifices, the neck and associated tissues and the cranial, thoracic, abdominal and pelvic cavities and viscera. External and cut surfaces of organs were examined as appropriate. Histopathology: adrenals, aorta, brain, caecum, colon, duodenum, epididymides, eye and optic nerve, femoral bone and stifle joint, gall bladder, heart, ileum, jejunum, kidneys, liver, lungs with mainstem bronchi, lymph nodes (mandibular, mesenteric), mammary gland (caudal), oesophagus, ovaries, pancreas, pituitary, prostate, rectum, salivary gland (submandibular), sciatic nerve, seminal vesicles, skeletal muscle (thigh), skin, spinal cord, spleen, sternum and marrow, stomach (keratinised, glandular), testes, thymus, thyroid with parathyroids, trachea, urinary bladder, uterus with cervix, vagina. Histopathological evaluation was performed on animals of all dose groups. All abnormal organs and tissues found at necropsy were also taken for histological examination.
3.5.3	Other examinations	None
3.5.4	Statistics	Mortality: Cox's test, Tarone's test, Kaplan-Meier method. Haematology: Mann-Whitney's test. Organ weights, food consumption and body weight changes: Bartlett's test, Behrens-Fisher test, Dunnett's test, as appropriate. Pathology/histopathology: Fisher's Exact test
3.6	**Further remarks**	

Section A6.5 **Carcinogenicity test in mice**
Annex Point IIA 6.5

4 RESULTS

4.1	**Observations**	
4.1.1	Clinical signs	In general, clinical signs of reactions to treatment were found in males of the 300 ppm group and comprised of thin build, ungroomed coats, hair loss and encrustations on the skin. Additionally, males at 100 ppm showed a slightly higher incidence of ungroomed coats in comparison to control. Isolated incidences of hunched posture were also observed in males at 300 ppm. Females were generally unaffected by treatment. However, a slightly increased incidence of over-activity was seen at 300 ppm. The group distribution, location, multiplicity and time of onset of the few palpable swellings was unaffected by treatment. Results are presented in Table A6.5-3.
4.1.2	Mortality	A total of 104 males and 61 females were killed or died during the study with an even distribution amongst the groups. Treatment of CD-1 mice with Alphacypermethrin did not adversely affect the survival of treated animals; indeed survival amongst females receiving 300 ppm was higher than that seen in controls. Results are presented in Table A6.5-3.
4.2	**Body weight**	Throughout the treatment period markedly lower body weight gains were seen in males and females receiving 300 ppm resulting in overall lower body weight gains of 26% and 24% than those of controls, respectively. A 13% lower body weight gain was observed in males of the 100 ppm group. Results are presented in Table A6.5-3.
4.3	**Food consumption and compound intake**	A marginal but insignificant group mean increase of food consumption was recorded for males at 300 ppm which was most apparent between weeks 14 and 26 of treatment. During the first 14 weeks of treatment the overall food conversion efficiency of males at 100 ppm and males and females at 300 ppm Alphacypermethrin was lower than that of the controls. The mean achieved dose levels were 3.0, 10.6 and 35.2 mg/kg bw/d for males and 3.5, 11.5 and 37.7 mg/kg bw/d for females treated with 30, 100 and 300 ppm Alphacypermethrin in the diet, respectively. Results are presented in Table A6.5-3.
4.4	**Blood analysis**	
4.4.1	Haematology	No treatment related changes were seen in blood smears evaluated after 50 or 77 weeks of treatment and in prematurely sacrificed animals.

Section A6.5 Carcinogenicity test in mice

Annex Point IIA 6.5

4.5	**Sacrifice and pathology**	
4.5.1	Organ weights	No inter-group differences in absolute and relative organ weights which were considered to be related to treatment with Alphacypermethrin were noted. A few statistically significant changes in high dose animals were attributed to the marked effect on terminal body weights.
4.5.2	Gross and histopathology	There were no macroscopic changes in animals killed after 52 and 78 weeks of treatment which were considered to be related to treatment. No treatment-related neoplastic or non-neoplastic findings were observed during histopathological examination. A few findings which achieved statistical significance were not attributed to treatment and considered to have arisen by chance.
4.6	**Other**	None

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Groups of 72 male and 72 female CD-1 mice received diets containing 0, 30, 100 and 300 ppm of Alphacypermethrin. 20 males and 20 females of each group were sacrificed for interim examination, after 52 weeks, the remainder were sacrificed after 78 weeks of treatment. The study was conducted according to OECD guideline 451 (1981).
5.2	**Results and discussion**	Administration of Alphacypermethrin to CD-1 mice for 78 weeks was associated with a number of non-specific effects. Treatment of animals with 300 ppm Alphacypermethrin resulted in poor growth performance and changes in appearance. In males, this was evident by a higher incidence of thin build during the in-life phase. Signs of reaction to treatment were, generally, confined to males receiving 300 ppm and included ungroomed coats, hair loss and surface encrustations on the skin and were probably related to the irritant properties of the test material. Other signs of reaction included occasional hunched posture in males and over-activity in females receiving 300 ppm. Overall weight gains of males and females in the 300 ppm group were 26 and 24 % lower than those of the controls and were associated with a reduced efficiency of food conversion. This is considered indicative of a non-specific toxicity rather than an influence of food intake. A similar, less marked effect on weight gain and food conversion efficiency was noted for males receiving 100 ppm. No treatment-related effects on haematology or organ weight changes were seen, and macroscopic and histopathological examinations revealed no changes attributable to treatment with the test substance. There was no evidence that Alphacypermethrin had any oncogenic potential at concentrations up to 300 ppm. Based on the poor growth performance and changes in appearance the NOAEL was determined to be 30 ppm.

Section A6.5 Carcinogenicity test in mice

Annex Point IIA 6.5

5.3	**Conclusion**	
5.3.1	LO(A)EL	100 ppm, corresponding to 10.8 mg/kg bw/d for male and 11.7 mg/kg bw/d for female mice. (conversion from "ppm in feed" to "mg/kg bw/d" calculated according to: LO(A)EL (ppm) x group mean food consumption / group mean body weight)
5.3.2	NO(A)EL	30 ppm, corresponding to 3.0 mg/kg bw/d for male and 3.5 mg/kg bw/d for female mice. (conversion from "ppm in feed" to "mg/kg bw/d" calculated according to: NO(A)EL (ppm) x group mean food consumption / group mean body weight)
5.3.3	Other	
5.3.4	Reliability	1
5.3.5	Deficiencies	No

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	April, 2009
Materials and methods	Applicant's version adopted
Results and discussion	Applicant's version adopted
Conclusion	Applicant's version adopted
Reliability	1
Acceptability	accepatble
Remarks	none
	COMMENTS FROM ...
Date	
Materials and methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.5- 2: Results of the 78-week feeding study in CD1-mice.

Parameter	Control		30 ppm		100 ppm		300 ppm		Dose-response +/–	
	m	f	m	f	m	f	m	f	m	f
No of animals examined										
Terminal phase: 78 weeks	52	52	52	52	52	52	52	52		
Interim phase: 52 weeks	20	20	20	20	20	20	20	20		
Mortality										
Terminal phase	22	21	22	11	21	20	26	7		
Interim phase	3	0	4	1	2	1	4	0		
Clinical signs										
Thin build[1]	3	18	4	18	8	21	19	25	+	+
Ungroomed coat[1]	9	4	7	1	18	3	55	5	+	
Hunched posture[1]	8	14	7	10	7	11	26	8	+	–
Over-activity[1]	0	5	1	6	1	4	2	12		+
Encrustations[1]	35	9	35	3	44	4	50	3	+	–
Hair loss (ventral)[2]	16/65	1/69	10/66	1/72	19/63	0/65	30/65	0/71	+	
Hair loss (dorsal)[2]	16/65	3/69	9/66	1/72	17/63	0/65	35/65	3/71	+	
Body weight gain [g]										
Weeks 0–26	27.3	17.3	26.7	13.8**	23.3**	15.6	20.7**	12.7**	–	–
Weeks 0–78	27.8	23.3	27.1	21.5	24.3	20.7	20.5**	17.8*	–	–
Food intake [g]										
Week 1–78	2795	2528	2802	2355	2817	2361	2922	2389	–	–
Food conversion efficiency [%][3]	4.6	2.7	4.5	2.4	3.7	2.8	3.2	2.2	–	–

* significantly greater or less than control mean ($p<0.05$)

** significantly greater or less than control mean ($p<0.01$)

[1] weeks 1–80 (total no. of animals affected)

[2] month 12 (maximum monthly incidence; no. of animals affected / no. of alive animals)

[3] weeks 1–14

Section A6.6.1 ***In-vitro* gene mutation study in bacteria**
Annex Point IIA 6.6

Official use only

1 REFERENCE

1.1	**Reference**	**A6.6.1/01:** Brooks T (1993) F TM: Bacterial mutagenicity studies. SRC, Sittingbourne, UK, Report no. SBTR.92.022, February 16, 1993 (unpublished). (BASF RDI No.: AL-435-005)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	No
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes OECD guideline 471 (1983) EU method B.13/14 (67/548/EC) The conduct of the study was consistent with EU method B.13/14 (2000/32/EC) in all important aspects.
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	FASTAC; Alphacypermethrin (WL085871), as given in Section A2
3.1.1	Lot/Batch number	02156; drum 1085
3.1.2	Specification	As given in Section A2
3.1.3	Purity	95.6%
3.1.4	Description	Off-white powder
3.1.5	Stability	The test substance was considered to be stable for the duration of the study.
3.2	**Study type**	Bacterial reverse mutation test
3.2.1	Organism/cell type	*S. typhimurium:* TA 1535, TA 1537, TA 98, TA 100, TA 1538 *E. coli:* WP2 uvr A (pKM101)

Section A6.6.1 ***In-vitro*** **gene mutation study in bacteria**
Annex Point IIA 6.6

3.2.2	Deficiencies / Proficiencies	No
3.2.3	Metabolic activation system	S9 mix: Rat liver homogenate from male Fischer 344 rats pre-treated with Aroclor 1254. A final concentration of 10% S9 in the S9 mix was used.
3.2.4	Positive control	With metabolic activation: *E.coli*, TA 1538, TA 98, TA 100: benzo(a)pyrene TA 1537: neutral red TA 1535: 2-aminoanthracene Without metabolic activation: *E.coli* potassium dichromate TA 1535, TA 100: sodium azide TA 1537: 9-aminoacridine TA 1538, TA 98: 2-nitrofluorene
3.3	**Application of test substance**	
3.3.1	Concentrations	31.25, 62.5, 125, 250, 500, 1000, 2000 or 5000 µg/plate (both in the presence and in the absence of rat liver S9 fraction)
3.3.1	Way of application	Plate incorporation assay: 20 µl of Alphacypermethrin in Acetone were added to the top agar mix and plated. The cultures were incubated at 37 °C for 48–72 hours.
3.3.2	Pre-incubation time	None
3.3.3	Other modifications	None
3.4	**Examinations**	Number of revertant colonies

4 RESULTS

4.1	**Genotoxicity**	
4.1.1	Without metabolic activation	Negative for induction of reverse mutation. Positive controls significantly increased the reverse mutation rate. The results are presented in Table A6.6.1- 1
4.1.2	With metabolic activation	Negative for induction of reverse mutation. Positive controls significantly increased the reverse mutation rate. The results are presented in Table A6.6.1- 1
4.2	**Cytotoxicity**	No

Section A6.6.1 ***In-vitro* gene mutation study in bacteria**
Annex Point IIA 6.6

		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The mutagenic potential of Alphacypermethrin was tested in the bacterial reverse mutation test using the plate incorporation assay. The method used was consistent with method B.13/14 (2000/32/EC) in all important aspects.
5.2	**Results and discussion**	Concentrations of up to 5000 µg Alphacypermethrin/plate were tested in the plate incorporation assay. No increase in the reverse gene mutation rate was found in any of the tested strains either in presence or absence of rat liver S9 fraction.
5.3	**Conclusion**	Alphacypermethrin was found to be not mutagenic under the conditions of the test.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	March 2009
Materials and methods	Applicant's version adopted
Results and discussion	APPLICANT'S VERSION ADOPTED
Conclusion	APPLICANT'S VERSION ADOPTED
Reliability	1
Acceptability	acceptable
Remarks	none
	COMMENTS FROM ...
Date	
Materials and methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.6.1- 1: Relative reverse mutation rates in *E. coli* or *S. typhimurium* after treatment with Alphacypermethrin (two plate-incorporation assays per concentration).

Concentration [µg per plate]	Relative reverse mutation rates[1]												Comments
	– S9						+ S9						
	WP_2	TA1535	TA1537	TA1538	TA98	TA100	WP_2	TA1535	TA1537	TA1538	TA98	TA100	
Control (mean number of revertant colonies)	77.3±13.1 74.0±3.6 85.7±7.6	13.0±2.6 9.0±1.0	10.0±3.0 8.3±1.2	11.3±1.5 11.7±2.9	14.7±3.8 17.3±5.9	74.7±9.1 101.3±11.1	82.3±7.2 70.3±2.1 101.3±5.9	12.7±1.5 10.0±1.7	11.0±1.0 10.7±3.8	13.0±3.0 11.7±2.9	14.7±4.2 23.7±3.5	89.3±2.3 115.3±15.6	
31.25	1.1 0.9 0.9	1.0 1.0	1.0 1.2	1.0 0.9	0.8 1.3	0.8 1.0	1.0 1.2 0.8	0.9 0.9	0.8 1.1	1.0 1.0	0.7 0.8	0.9 1.0	No evidence of cytotoxicity was observed, on microscopical examination of the background lawn, at any amount tested.
62.5	1.1 0.8 1.2	0.8 1.0	1.1 1.2	1.1 1.0	0.7 0.9	0.9 1.1	1.1 1.2 1.0	0.8 1.0	0.9 1.3	1.0 1.1	0.9 0.8	0.8 1.1	
125	1.1 1.0 1.1	1.0 1.2	1.0 1.1	0.8 0.8	0.9 0.9	0.9 1.0	1.3 1.2 1.1	0.9 1.0	0.8 0.8	0.9 1.1	0.8 0.9	0.8 1.0	
250	1.2 0.9 1.2	0.9 0.9	1.0 1.1	0.8 0.8	0.7 1.2	0.9 0.9	1.0 1.1 1.2	1.1 0.8	1.0 0.8	0.9 1.1	1.0 0.8	0.8 0.8	
500	1.2 0.9 1.1	1.0 1.1	1.1 0.9	1.1 0.8	0.6 0.9	0.9 1.0	1.1 1.2 1.4	0.8 1.0	0.9 1.1	1.0 1.0	0.9 0.9	0.9 1.0	
1000	1.2 1.1 1.1	0.8 0.7	1.0 1.2	1.1 0.9	0.8 0.9	0.9 1.0	1.3 1.2 1.3	0.9 0.8	1.1 1.1	0.9 1.2	1.1 0.9	0.9 0.9	
2000	1.3 0.9 1.1	0.8 1.0	0.7 1.0	1.0 0.9	0.6 0.8	1.0 1.0	1.3 1.2 1.2	0.9 0.9	0.8 0.9	1.1 1.1	0.9 1.1	1.0 1.0	
5000	1.2 0.9 1.2	1.0 1.1	0.8 1.0	1.0 0.9	0.9 0.9	1.0 1.0	1.3 1.2 1.3	1.1 1.0	1.0 1.0	0.8 1.1	0.9 0.8	0.9 1.0	

(continued on next page)

Table A6.6.1- 1: Relative reverse mutation rates in *E. coli* or *S. typhimurium* after treatment with Alphacypermethrin (two plate-incorporation assays per concentration).

Positive controls [µg per plate]												
Sodium azide (2 µg)	–	72.4*	–	–	–	–	–	–	–	–	–	–
	–	81.6*	–	–	–	–	–	–	–	–	–	–
Sodium azide (5 µg)	–	–	–	–	–	10.0*	–	–	–	–	–	–
	–	–	–	–	–	11.1*	–	–	–	–	–	–
9-Aminoacridine (50 µg)	–	–	25.5*	–	–	–	–	–	–	–	–	
	–	–	31.1*	–	–	–	–	–	–	–	–	–
2-Aminoanthracene (5 µg)	–	–	–	–	–	–	–	12.5*	–	–	–	
	–	–	–	–	–	–	–	29.4*	–	–	–	–
2-Nitrofluorene (5 µg)	–	–	–	102.7*	60.2*	–	–	–	–	–	–	
	–	–	–	79.7*	46.7*		–	–	–	–	–	–
Benzo(a)pyrene (10 µg)	–	–	–	–	–	–	6.3*	–	–	14.7*	17.0*	5.2*
	–	–	–	–	–	–	7.5*	–	–	18.1*	12.4*	6.4*
	–	–	–	–	–	–	7.9*					
K-dichromate (20 mg)	8.5*	–	–	–	–	–	–	–	–	–	–	–
	4.9*	–	–	–	–	–	–	–	–	–	–	–
	7.6*											
Neutral red (20 mg)	–	–	–	–	–	–	–	–	13.7*	–	–	–
	–	–	–	–	–	–	–	–	12.9*	–	–	–

1 results are expressed as ratio: Mean number of revertant colonies per treated plate / Mean number of revertant colonies per control plate
– not tested
* Reproducible values of 2.5 × control value or greater were considered to indicate mutagenic response.

Section A6.6.1 *In vitro* gene mutation study in yeast

Annex Point IIA6.6 **Supportive data**

The following reference is considered to contain additional information concerning genotoxicity of Alphacypermethrin and is thus presented in an abbreviated format (adopted from the PPP-dossier) as supportive data:

Reference: **A6.6.1/02**

Brooks T (1984) Genotoxicity studies with Fastac; the induction of gene mutation in the yeast *Saccharomyces cerevisiae* XV185-14C. SRC, Sittingbourne, UK, Report no. SBGR.84.117, April 1984 (unpublished), BASF RDI No.: AL-435-002.

Guidelines: Non-guideline study.

GLP: No

System:

Saccharomyces cerevisiae XV185-14C (arg 4-17, trp 5-48 and hom 3-10 loci)

Concentrations of 31.25, 62.5, 125, 250, 500, 1000, 2000 and 4000 µg/ml Alphacypermethrin were tested.

Findings:

Alphacypermethrin did not increase the reverse gene mutation rate at each of the four loci with and without S9. Positive controls cyclophosphamide and 4-nitroquinoline-N-oxide significantly induced reverse gene mutation.

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

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	March 2009
Materials and Methods	Applicant's version adopted
Results and discussion	APPLICANT'S VERSION ADOPTED
Conclusion	Alphacypermethrin was found to be not mutagenic under the conditions of the test.
Reliability	2 (no guideline)
Acceptability	acceptable
Remarks	none

	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.6.2 ***In-vitro* cytogenicity study in mammalian cells**
Annex Point IIA 6.6

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1 REFERENCE

1.1	**Reference**	**A6.6.2/01:** Brooks T, Wiggins D (1993) Fastac TM: *In vitro* chromosome studies using cultured human lymphocytes. SRC, Sittingbourne. UK, Report no. SBTR.93.007, April 27, 1993 (unpublished). (BASF RDI No.: AL-435-006)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	No
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes OECD guideline 473 (1983) The conduct of the study was consistent with method B.10 (2000/32/EC).
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	FASTAC, Alphacypermethrin (WL085871), as given in section A2
3.1.1	Lot/Batch number	02156; drum 1085
3.1.2	Specification	As given in Section A2
3.1.3	Purity	95.6%
3.1.4	Description	Off-white powder
3.1.5	Stability	The test substance was considered to be stable for the duration of the study.
3.2	**Study type**	*In vitro* mammalian chromosome aberration test
3.2.1	Organism/cell type	Human lymphocytes
3.2.2	Deficiencies/ Proficiencies	None
3.2.3	Metabolic activation system	S9 fraction from Aroclor 1254 induced rat liver. The S9-mix was added to the cell cultures with a final concentration of 5%.

Section A6.6.2 ***In-vitro*** **cytogenicity study in mammalian cells**
Annex Point IIA 6.6

3.2.4	Positive control	Cyclophosphamide (with S9), Mitomycin C (without S9)
3.3	**Application of test substance**	
3.3.1	Concentrations	1st experiment: 125, 500 and 1000 µg/ml (24 h sampling time) 2nd experiment: 93.75, 375, 500 and 750 µg/ml (24 h sampling time) 3rd experiment: 1000 µg/ml (48 h sampling time) The highest concentration considered suitable was 1000 µg/ml; higher concentrations formed a white precipitate in the test medium.
3.3.2	Way of application	The test compound was dissolved in Acetone and 0.025 ml was added to the whole blood cultures. In the presence of S9, the exposure time for the test compound was 3 hours, and cells were sampled after 24 and 48 hour of exposure. In the absence of S9, cells were continuously exposed to treatment medium for 24 and 48 hours. The cultures were incubated at 37°C.
3.3.3	Pre-incubation time	None
3.3.4	Other modifications	None
3.4	**Examinations**	Evaluation of chromosome or chromatid aberrations
3.4.1	Number of cells evaluated	Where possible 200 metaphases were scored for each dose group. Only those cells showing the modal chromosome number (46) ± 2 centromeres were analysed for chromosome damage. The mitotic index (MI) was assessed by counting the number of metaphases in a total of 1000 cells and the mitotic index was calculated.

4 RESULTS

4.1	**Genotoxicity**	
4.1.1	Without metabolic activation	Negative Human lymphocytes exposed to Alphacypermethrin concentrations up to 1000 µg/ml for 24 hours showed no increase in structural chromosome damage when compared to the control in two replicate experiments (cyt 981 and cyt 993). After exposure for 48 hours at 1000 mg/ml, no increase in any type of aberrations over the control was seen. The positive control compound, Mitomycin C, showed significantly more cells with aberrations excluding and including gaps and chromatid gaps and/or isogaps than the solvent control in each experiment. The results are summarised in Table A6.6.2-1 and Table A6.6.2- 3 and Table A6.6.2- 4.

Section A6.6.2 ***In-vitro*** **cytogenicity study in mammalian cells**
Annex Point IIA 6.6

4.1.2	With metabolic activation	Two replicate experiments were conducted exposing human lymphocytes to Alphacypermethrin for 3 hours in the presence of S9 mix. In the first experiment (cyt 979), small statistically significant differences in the number of cells with chromatid aberrations (gaps and/or isogaps) were observed in the highest concentration. A second experiment (cyt 989) showed no significant treatment-related differences. Therefore, the small increase seen in chromatid gaps and/or isogaps was considered to be of no biological significance. Exposure of human lymphocyte (1000 µg/ml) for 3 hours and harvest after further 45 hours did not result in statistically significant differences compared to solvent control (cyt 990). The positive control, Cyclophosphamide, showed significantly more cells with structural chromosome aberrations than the solvent control in each experiment. The results are summarised in Table A6.6.2- 5 and Table A6.6.2- 6 and Table A6.6.2- 7.
4.2	**Cytotoxicity**	No cytotoxicity was observed at concentrations up to 1000 µg/ml Alphacypermethrin in the presence and absence of S9 mix. The results are summarised in Table A6.6.2- 5 and Table A6.6.2- 6 and Table A6.6.2- 7.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The *in-vitro* genotoxicity of Alphacypermethrin was tested in human lymphocytes according to the OECD guideline 473 (1983). In all important aspects the study was consistent to method B.10 (2000/32/EC).
5.2	**Results and discussion**	Alphacypermethrin did not induce structural chromosome damage in cultured human lymphocytes either in the presence or in the absence of S9 mix.
5.3	**Conclusion**	Under the conditions of the test, Alphacypermethrin was considered not genotoxic.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	March 2009
Materials and methods	Applicant's version adopted
Results and discussion	APPLICANT'S VERSION ADOPTED
Conclusion	APPLICANT'S VERSION ADOPTED
Reliability	1
Acceptability	acceptable
Remarks	none
	COMMENTS FROM ...
Date	
Materials and methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.6.2- 1: Metaphase chromosome analysis of cultured human lymphocytes (cyt 981) after a 24 hour exposure to Alphacypermethrin or Mitomycin C (MMC) in the absence of S9 mix.

	Control	**Solvent control**	**125 µg/ml**	**500 µg/ml**	**1000 µg/ml**	**0.2 µg/ml MMC**
No. of cells analysed	**200**	**200**	**200**	**200**	**200**	**200**
Cytotoxicity	No	No	No	No	No	No
Polyploid cells [%]	0.5	0	0	0	0	0
Excluding gaps						
Sum total of aberrations	0	0	0	0	0	58
Mean of aberrations/cell	0	0	0	0	0	0.29
No. cells with aberrations	0	0	0	0	0	42**
Cells with aberrations [%]	0	0	0	0	0	21.00
Including gaps						
Sum total of aberrations	3	1	0	0	1	80
Mean of aberrations/cell	0.015	0.005	0	0	0.005	0.400
No. cells with aberrations	3	1	0	0	1	52**
Cells with aberrations [%]	1.51	0.50	0	0	0.50	26.00
Mitotic index[1] [%]	95	100	79	144	87	–

** statistically significant increase compared

–) Not stated in the report.

1) Mitotic index relative to the solvent control.

Table A6.6.2- 2: Metaphase chromosome analysis of cultured human lymphocytes (cyt 993) after a 24 hour exposure to Alphacypermethrin or Mitomycin C (MMC) in the absence of S9 mix.

	Control	Solvent Control	93.75 µg/ml	375 µg/ml	500 µg/ml	750 µg/ml	0.2 µg/ml MMC
No. of cells analysed	**200**	**200**	**200**	**200**	**194**	**200**	**78**
Cytotoxicity	No	No	No	No	No	No	No
Polyploid cells [%]	0	0	0	0	0	0	0
Excluding gaps							
Sum total of aberrations	2	0	1	0	1	0	36
Mean of aberrations/cell	0.010	0	0.005	0	0.005	0	0.462
No. cells with aberrations	2	0	1	0	1	0	26
Cells with aberrations [%]	1.00	0	0.50	0	0.52	0	33.33
Including gaps							
Sum total of aberrations	4	1	3	2	5	4	65
Mean of aberrations/cell	0.020	0.005	0.015	0.010	0.026	0.020	0.833
No. cells with aberrations	4	1	3	2	4	4	38
Cells with aberrations [%]	2.00	0.50	1.50	1.00	2.06	2.00	48.72

Table A6.6.2- 3: Metaphase chromosome analysis of cultured human lymphocytes (cyt 992) after a 48 hour exposure to Alphacypermethrin in the absence of S9 mix.

	Untreated control	Solvent control	1000 µg/ml
No. of cells analysed	**200**	**200**	**200**
Cytotoxicity	No	No	No
Polyploid cells [%]	1.00	0.50	0
Excluding gaps			
Sum total of aberrations	1	0	3
Mean of aberrations/cell	0.005	0	0.015
No. cells with aberrations	1	0	1
Cells with aberrations [%]	0.50	0	0.50
Including gaps			
Sum total of aberrations	1	1	5
Mean of aberrations/cell	0.005	0.005	0.025
No. cells with aberrations	1	1	3
Cells with aberrations [%]	0.50	0.50	1.50
Mitotic index[1] [%]	132	100	91

1) Mitotic index relative to the control cultures

Table A6.6.2- 4: Metaphase chromosome analysis of cultured human lymphocytes (cyt 979) after a 3 hour exposure to Alphacypermethrin or Cyclophosphamide (CP) in the presence of S9 mix, sample time 24 hours.

	Control	Solvent Control	125 µg/ml	500 µg/ml	1000 µg/ml	10 µg/ml CP
No. of cells analysed	**200**	**200**	**200**	**200**	**200**	**200**
Cytotoxicity	No	No	No	No	No	No
Polyploid cells [%]	0	0.50	0	0	0	0.50
Excluding gaps						
Sum total of aberrations	0	0	1	1	0	103
Mean of aberrations/cell	0	0	0.005	0.005	0	0.518
No. cells with aberrations	0	0	1	1	0	64
Cells with aberrations [%]	0	0	0.50	0.50	0	32.16
Including gaps						
Sum total of aberrations	2	0	4	1	6	177
Mean of aberrations/cell	0.010	0	0.020	0.005	0.030	0.889
No. cells with aberrations	2	0	4	1	5	93
Cells with aberrations [%]	1.00	0	2.00	0.50	2.50	46.73
Mitotic index[1] [%]	95	100	86	83	71	–

–) Not stated in the report.

1) Mitotic index relative to the solvent control.

Table A6.6.2- 5: Metaphase chromosome analysis of cultured human lymphocytes (cyt 989) after a 3 hour exposure to Alphacypermethrin or Cyclophosphamide (CP) in the presence of S9 mix, sample time 24 hours.

	Control	Solvent control	125 µg/ml	500 µg/ml	1000 µg/ml	10 µg/ml CP
No. of cells analysed	**200**	**200**	**200**	**200**	**200**	**200**
Cytotoxicity	No	No	No	No	No	No
Polyploid cells [%]	0	0	1.00	0	0	0
Excluding gaps						
Sum total of aberrations	0	0	2	1	1	83
Mean of aberrations/cell	0	0	0.010	0.005	0.005	0.415
No. cells with aberrations	0	0	2	1	1	52
Cells with aberrations [%]	0	0	1.01	0.50	0.50	26.00
Including gaps						
Sum total of aberrations	0	0	4	2	1	108
Mean of aberrations/cell	0	0	0.020	0.010	0.005	0.540
No. cells with aberrations	0	0	4	2	1	64
Cells with aberrations [%]	0	0	2.02	1.00	0.50	32.00

Table A6.6.2- 6: Metaphase chromosome analysis of cultured human lymphocytes (cyt 990) after a 3 hour exposure to Alphacypermethrin in the presence of S9 mix, sample time 48 hours.

	Untreated control	**Solvent control**	**1000 µg/ml**
No. of cells analysed	**200**	**200**	**200**
Cytotoxicity	No	No	No
Polyploid cells [%]	0	0	0
Excluding gaps			
Sum total of aberrations	0	0	0
Mean of aberrations/cell	0	0	0
No. cells with aberrations	0	0	0
Cells with aberrations [%]	0	0	0
Including gaps			
Sum total of aberrations	3	1	1
Mean of aberrations/cell	0.015	0.005	0.005
No. cells with aberrations	3	1	1
Cells with aberrations [%]	1.50	0.50	0.50
Mitotic index[1] [%]	100	100	106

1) Mitotic index relative to the control cultures.

Section A6.6.3 ***In-vitro* gene mutation study in mammalian cells**
Annex Point IIA 6.6

Official use only

1 REFERENCE

1.1	**Reference**	**A6.6.3/01:** van de Waart EJ (1994) Evaluation of the mutagenic activity of Fastac technical in an in vitro mammalian cell gene mutation test with L5178Y mouse lymphoma cells (with independent repeat). Notox B.V., 's-Hertogenbosch, Netherlands, Report no. 087367, December 21, 1994 (unpublished), BASF RDI No.: AL-435-007.
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	No
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes OECD guideline 476 (1984) EEC publication no. L133 (1988) U.S. EPA Subdivision F, Guideline Nos. 84-1 & 84-2 The conduct of the study was consistent with method B.17 (2000/32/EC) in all important aspects.
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	FASTAC technical, synonym for Alphacypermethrin, as given in Section A2
3.1.1	Lot/Batch number	ST91/243
3.1.2	Specification	As given in Section A2
3.1.3	Purity	95.4%
3.1.4	Description	Off-white powder
3.1.5	Stability	It is stated that the test substance was stable under storage conditions.
3.2	**Study type**	*In vitro* mammalian cell gene mutation test
3.2.1	Organism/cell type	L5178Y mouse lymphoma cells

Section A6.6.3 ***In-vitro*** **gene mutation study in mammalian cells**
Annex Point IIA 6.6

3.2.2	Deficiencies/ Proficiencies	Thymidine kinase (TK) deficient cells, due to the forward mutation (TK+/– to TK–/–).
3.2.3	Metabolic activation system	S9 mix Rat liver microsomal enzymes were routinely prepared from adult male Wistar or Sprague Dawley rats, which were injected intraperitoneally with a solution of Aroclor 1254 in corn oil.
3.2.4	Positive control	Without S9 mix: Ethylmethanesulphonate (EMS) With S9 mix: Dimethylnitrosamine (DMN)
3.3	**Application of test substance**	
3.3.1	Concentrations	3.3, 10, 33, 50 µg/mL and solvent control (DMSO) The highest concentration considered suitable was 50 µg/mL; higher concentrations formed a precipitate in the test medium.
3.3.2	Way of application	The test compound was formulated in dimethyl sulphoxide (DMSO) and tested in two independent experiments. Cells were exposed for 2 hours to the test substance and subsequently sub-cultured for 3 days for expression of the mutant phenotype. After the expression period cells were plated with selective medium for 10–14 days. Additionally, cells were plated for determination of cell survival (cloning efficiency).
3.3.3	Pre-incubation time	None
3.3.4	Other modifications	None
3.4	**Examinations**	The effect of the test substance on the frequency of mutation was tested by determination of the resistance of TK deficient cells to the cytotoxic effects of the pyrimidine analogue trifluorothymidine (TFT).
3.4.1	Number of cells evaluated	1.5×10^6 on ten plates per concentration level; mutant frequency expressed as number of mutants per 10^5 surviving cells.

4 RESULTS

4.1	**Genotoxicity**	
4.1.1	Without metabolic activation	Negative In the absence of S9 mix the test substance did not induced significant increases in the mutant frequency in both independent experiments. Mutant frequencies induced by EMS increased by 9–24-fold. Results are presented in Table A6.6.3- 1.

Section A6.6.3 ***In-vitro* gene mutation study in mammalian cells**
Annex Point IIA 6.6

4.1.2	With metabolic activation	Negative A slight increase in the mutant frequency at the highest test concentration was observed in one experiment. However, this was considered not treatment-related, because the finding was not dose-related, within the historical control data range and not reproducible in the second experiment. Mutant frequencies induced by DMN increased by 15 to 23-fold. Results are presented in Table A6.6.3- 2.
4.2	**Cytotoxicity**	Negative Concentrations up to 50 µg/ml Alphacypermethrin showed no significant inhibition of the growth of the lymphoma cells. But precipitation in the exposition medium at concentration from 50 µg/ml and upwards were observed.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The *in-vitro* genotoxicity of Alphacypermethrin was tested in mouse lymphoma cells (L5178Y). The study was conducted according OECD guideline 476 (1984), EEC publication no. L133 (1988) and U.S. EPA Subdivision F, Guideline Nos. 84-1 & 84-2. The method used was consistent in all important aspects to method B.17 (2000/32/EC).
5.2	**Results and discussion**	In the presence of S9 mix, Alphacypermethrin induced a slight increase in the mutant frequency at the highest test substance concentration in one experiment only, but this finding was considered not treatment-related. In the absence of S9 mix, Alphacypermethrin induced no significant increase in the mutant frequency in both independent experiments.
5.3	**Conclusion**	It was concluded that Alphacypermethrin was **non mutagenic** under the conditions of this test.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

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

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	March, 2009
Materials and Methods	Applicant's version adopted
Results and discussion	Applicant's version adopted
Conclusion	Applicant's version adopted
Reliability	2 because of shorte exposure time compared to guidelines
Acceptability	acceptable
Remarks	none

	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.6.3- 1: The effect of Alphacypermethrin or Ethylmethanesulphonate (EMS) on forward mutation rates to TFT-resistance in L5178Y mouse lymphoma cells in absence of metabolic activation on day 3.

Concentration [mg/ml]	– S9				Historical control
	Experiment 1		Experiment 2		
	Mutation frequency (x 10^5 cells)	Mean plating efficiency %	Mutation frequency (x 10^5 cells)	Mean plating efficiency %	Mutation frequency (x 10^5 cells)
0	0.8	79	0.8	91	1.9 ± 1.1
3.3	0.7	78	0.7	91	–
10	0.9	74	0.9	83	–
33	0.8	82	1.2	96	–
50[1]	1.5	51	1.0	98	–
2 mM EMS	7.5	80	19.0	64	–

1) test substance precipitated slightly in exposure medium.

Table A6.6.3- 2: The effect of Alphacypermethrin or Dimethylnitrosamine (DMN) on forward mutation rates to TFT resistance in L5178Y mouse lymphoma cells in presence of metabolic activation on day 3.

Concentration [mg/ml]	+ S9				Historical control
	Experiment 1		Experiment 2		
	Mutation frequency (x 10^5 cells)	Mean plating efficiency %	Mutation frequency (x 10^5 cells)	Mean plating efficiency %	Mutation frequency (x 10^5 cells)
0	1.3	81	0.7	86	1.6 ± 0.8
3.3	1.1	82	0.7	70	–
10	0.8	89	0.7	94	–
33	1.5	81	0.7	91	–
50[1]	1.7	73	1.7	77	–
0.5 mM DMN	19.3	83	16.2	40	–

1) test substance precipitated slightly in exposure medium

Section A6.6.4 **Genotoxicity *in vivo***
Annex Point IIA 6.6 **– mammalian bone marrow micronucleus test –**

Official use only

1 REFERENCE

1.1	**Reference**	**A6.6.4/01:** [redacted] (1995) Micronucleus test in bone marrow cells of the mouse with Fastac technical. [redacted], Report no. 087378 (unpublished). (BASF RDI No.: AL-435-008)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	No
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes OECD guideline 474 (1983) EEC Directive 84/449 Annex V, B.12 (1984) EPA test guidelines, Subchapter R, part 798, subpart F (1989) The conduct of the study was consistent with EU method B.12 (2000/32/EC) in all important aspects.
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	FASTAC technical, synonym for Alphacypermethrin, as given in Section A2.
3.1.1	Lot/Batch number	ST91/243
3.1.2	Specification	Not stated
3.1.3	Purity	95.4%
3.1.4	Description	Off-white powder
3.1.5	Stability	It is stated that the test substance was stable under storage conditions.
3.1.6	Maximum tolerable dose	A dose of 10 mg/kg bw was considered to be the maximum tolerable dose over 48 to 72 hours, based on a preliminary toxicity test.
3.2	**Test animals**	
3.2.1	Species	Mouse

Section A6.6.4 **Genotoxicity *in vivo***
Annex Point IIA 6.6 **– mammalian bone marrow micronucleus test –**

3.2.2	Strain	Swiss, OF-1 (SPF-quality)	
3.2.3	Source	BRL, Switzerland	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	Age: Approx. 6 weeks. Body weight: 21–27 g (females) 29–37 g (males)	
3.2.6	Number of animals per group	5 males and 5 females, per dose and sampling time (24, 48 and 72 h)	
3.2.7	Control animals	Yes 5 males and 5 females (vehicle control) per sampling time (24, 48 and 72 h); 5 males and 5 females (positive control) per sampling time (24 h)	
3.3	**Administration/ Exposure**		
3.3.1	Number of applications	Single	
3.3.2	Interval between applications	Not applicable	
3.3.3	Post-exposure period	24, 48 or 72 hours	
3.3.4	Type	Oral by gavage	
3.3.5	Dose	1, 5 and 10 mg/kg bw	
3.3.6	Vehicle	Corn oil	
3.3.7	Concentration in vehicle	1 mg/ml	
3.3.8	Total volume applied	1, 5 or 10 ml/ kg bw	
3.3.9	Controls	Corn oil (vehicle control) Cyclophosphamide (positive control)	
3.4	**Examinations**		
3.4.1	Clinical signs	Yes (daily)	
3.4.2	Tissue	Bone marrow	
		Number of animals:	5 per sex at each of 3 time points
		Number of cells:	1000 polychromatic erythrocytes (PCE)
		Time points:	24, 48 and 72 hours after treatment
		Type of cells:	polychromatic erythrocytes (PCE) / normochromatic erythrocytes (NCE)
		Parameters:	number of micronucleated polychromatic erythrocytes; PCE/NCE ratio.
3.5	**Further remarks**	None	

Section A6.6.4 **Genotoxicity *in vivo***
Annex Point IIA 6.6 **– mammalian bone marrow micronucleus test –**

4 RESULTS

4.1	**Clinical signs**	No animals died during the course of the study.
4.2	**Haematology/ Tissue examination**	The ratio of PCE/NCE was not affected by treatment with Alphacypermethrin. Cyclophophamide decreased the ratio of PCE/NCE. Results are presented in Table A6.6.4- 1 and Table A6.6.4- 2.
4.3	**Genotoxicity**	No increase in the frequency of micronucleated polychromatic erythrocytes was observed in the bone marrow of animals of both sex treated with Alphacypermethrin. In the positive control group, a statistically significant increase in the number of micronucleated PCEs was seen. Results are presented in Table A6.6.4- 1 and Table A6.6.4- 2.
4.4	**Other**	None

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The effect of Alphacypermethrin on the frequency of micronucleated polychromatic erythrocytes as an indication for induction of chromosome damage was investigated in mice. The test method was based on OECD 474 (1983) EEC Directive 84/449 Annex V, B.12 (1984) and EPA test guidelines, Subchapter R, part 798, subpart F (1989). The conduct of the study was consistent with EU method B.12 (2000/32/EC) in all important aspects.
5.2	**Results and discussion**	Single oral treatment of male and female mice with Alphacypermethrin at 1, 5 or 10 mg/kg bw did not cause any increase of micronucleated polychromatic erythrocytes in bone marrow at any of the three sampling times at either dose level.
5.3	**Conclusion**	Alphacypermethrin was not genotoxic under the conditions of the test.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

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

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	March, 2009
Materials and Methods	Applicant's version adopted
Results and discussion	Applicant's version adopted
Conclusion	Applicant's version adopted
Reliability	1
Acceptability	acceptable
Remarks	none

	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.6.4- 1: Mean number of micronucleated polychromatic erythrocytes per 1000 polychromatic erythrocytes and ratio of polychromatic/normochromatic erythrocytes in males.

Dose [mg/kg bw]	Sampling time [h]	No. of micronucleated PCEs per 1000 PCEs (mean ± S.D.)	Ratio PCE / NCE (mean ± S.D.)
0	24	0.6 ± 0.5	1.01 ± 0.07
0	48	0.4 ± 0.9	0.99 ± 0.05
0	72	0.2 ± 0.4	0.95 ± 0.09
1	24	0.8 ± 0.4	1.05 ± 0.08
1	48	0.4 ± 0.5	1.00 ± 0.08
1	72	0.0 ± 0.0	1.01 ± 0.07
5	24	0.6 ± 0.9	1.04 ± 0.02
5	48	0.4 ± 0.5	1.00 ± 0.01
5	72	0.4 ± 0.5	1.01 ± 0.03
10	24	0.6 ± 0.9	0.99 ± 0.04
10	48	0.4 ± 0.9	0.96 ± 0.05
10	72	0.6 ± 0.5	0.99 ± 0.04
50 CP	48	16.0 ± 4.9*	0.39 ± 0.07

CP Cyclophosphamide

* significantly different from corresponding control group (Wilcoxon Rank Sum Test, $P \leq 0.05$)

Table A6.6.4- 2: Mean number of micronucleated polychromatic erythrocytes per 1000 polychromatic erythrocytes and ratio of polychromatic/normochromatic erythrocytes in females.

Dose [mg/kg bw]	Sampling time (hours)	No. of micronucleated PCEs per 1000 PCEs (mean ± S.D.)	Ratio PCE / NCE (mean ± S.D.)
0	24	1.2 ± 1.3	1.06 ± 0.02
0	48	0.8 ± 0.8	1.00 ± 0.03
0	72	0.4 ± 0.5	1.04 ± 0.08
1	24	0.6 ± 0.5	1.04 ± 0.04
1	48	0.2 ± 0.4	1.02 ± 0.07
1	72	0.0 ± 0.0	1.06 ± 0.02
5	24	1.2 ± 0.8	1.05 ± 0.03
5	48	0.6 ± 0.9	1.01 ± 0.06
5	72	0.2 ± 0.4	1.05 ± 0.02
10	24	0.4 ± 0.5	1.04 ± 0.03
10	48	0.8 ± 0.8	1.02 ± 0.04
10	72	0.2 ± 0.4	1.02 ± 0.04
50 CP	48	8.6 ± 2.6*	0.46 ± 0.17

CP Cyclophosphamide

* significantly different from corresponding control group (Wilcoxon Rank Sum Test, $P \leq 0.05$)

Section A6.6.4 **Genotoxicity *in vivo***
Annex Point IIA 6.6 **– *in vivo* mammalian chromosome aberration test –**

Official use only

1 REFERENCE

1.1	**Reference**	**A6.6.4/02:** [redacted] (1984) Genotoxicity studies with Fastac: *In vivo* cytogenetic test using rat bone marrow. [redacted], Report no. SBGR.84.120, May 09, 1984 unpublished. (BASF RDI No.: AL-435-003)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	No
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No The conduct of the study was consistent with method B.11 (2000/32/EC) in all important aspects.
2.2	**GLP**	No
2.3	**Deviations**	Not applicable

3 MATERIALS AND METHODS

3.1	**Test material**	FASTAC technical, Alphacypermethrin, as given in Section A2.
3.1.1	Lot/Batch number	ST84/007; F830047B, RS0016/84
3.1.2	Specification	Not stated
3.1.3	Purity	95.8%
3.1.4	Description	White powder
3.1.5	Stability	It is stated that the test substance was stable under storage conditions.
3.1.6	Maximum tolerable dose	Because single oral doses of 10, 20 or 40 mg/kg bw of Alphacypermethrin proved to be toxic, a dose of 8 mg/kg bw was considered to be the maximum tolerable dose.
3.2	**Test animals**	
3.2.1	Species	Rat
3.2.2	Strain	Wistar, Charles River, pathogen-free
3.2.3	Source	Charles River, Manston, GB

Section A6.6.4 **Genotoxicity *in vivo***
Annex Point IIA 6.6 **– *in vivo* mammalian chromosome aberration test –**

3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	Age: 8–9 weeks. Body weight: 180–350g	
3.2.6	Number of animals per group	6 males and 6 females per dose	
3.2.7	Control animals	Yes 7 males and 7 females per group (positive and negative controls)	
3.3	**Administration/ Exposure**		
3.3.1	Number of applications	Single	
3.3.2	Interval between applications	Not applicable	
3.3.3	Post-exposure period	24 hours	
3.3.4	Type	Oral by gavage	
3.3.5	Dose	2, 4 and 8 mg/kg bw	
3.3.6	Vehicle	Corn oil	
3.3.7	Concentration in vehicle	5% w/v	
3.3.8	Total volume applied	0.04, 0.08 or 0.16 ml/kg bw	
3.3.9	Controls	Corn oil (vehicle control) Cyclophosphamide (positive controls: 25 mg/kg bw i.v. and 40 mg/kg bw p.o.)	
3.4	**Examinations**		
3.4.1	Clinical signs	Yes	
3.4.2	Tissue	Femur bone marrow	
		Number of animals:	at least 5 males and 5 females
		Number of cells:	at least 500 cells from each animal for mitotic index (MI); up to 200 cells for aberrations or polyploidy
		Time point:	24 hours after treatment
		Parameters:	polyploidy, chromatid aberrations, chromosome aberrations, severe damage.
3.5	**Further remarks**	None	

Section A6.6.4 **Genotoxicity *in vivo***
Annex Point IIA 6.6 **– *in vivo* mammalian chromosome aberration test –**

4 RESULTS

4.1	**Clinical signs**	Initially, doses of 10, 20, and 40 mg/kg Alphacypermethrin were used, but animals exhibited severe signs of toxicity (all male rats died) and the experiment was terminated. In the final experiment, one female exposed to 8 mg/kg Alphacypermethrin died shortly after dosing. All animals treated with Alphacypermethrin showed evidence of facial irritation shortly after dosing. Other symptoms, particularly common in animals exposed to 8 mg/kg or more, included hypersensitivity to noise and touch, pilo-erection, blood around the nose and mouth, salivation, swollen faces, hind leg paralysis with a splayed hind leg gait and subdued or semi-comatose behaviour.
4.2	**Haematology/ Tissue examination**	Male rats treated with 8 mg/kg Alphacypermethrin showed a reduction of the mitotic index (MI) to one half of the control. However Alphacypermethrin did not appear to affect the proportion of dividing cells in the bone marrow of females, even in those rats exposed to 10, 20 or 40 mg/kg. Cyclophosphamide depressed the MI more markedly at 40 mg/kg b.w. p.o. than at 25 mg/kg b.w. i.p. Results are presented in Table A6.6.4- 3.
4.3	**Genotoxicity**	Alphacypermethrin did not significantly increase either the frequency of cells showing any structural aberrations or the frequency of chromatid and chromosome breaks and exchanges in either the males or females at 2, 4 or 8 mg/kg. There was no evidence that Alphacypermethrin induced any type of aberration in the females that survived 10, 20 or 40 mg/kg. Cyclophosphamide significantly increased the frequency of cells showing structural aberrations and the frequency of chromatid/chromosome breaks and exchanges. Results are presented in Table A6.6.4- 3.
4.4	**Other**	None

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The effect of Alphacypermethrin on the incidence of chromosomal damage was tested in rat bone marrow following single oral administration of 2, 4 or 8 mg/kg b.w. Although not a guideline study the conduct was consistent with method B.11 (2000/32/EC) in all important aspects.
5.2	**Results and discussion**	Single oral treatment of male and female rats with Alphacypermethrin at 2, 4 or 8 mg/kg b.w. did not induce any statistically significant increase in the incidence of chromatid or chromosome aberrations or polyploidy in bone marrow cells compared with the incidence in animals dosed with corn oil.

Section A6.6.4 **Genotoxicity *in vivo***
Annex Point IIA 6.6 **– *in vivo* mammalian chromosome aberration test –**

5.3	**Conclusion**	Alphacypermethrin was not genotoxic under the conditions of the test.	
5.3.1	Reliability	2	
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities		
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	March, 2009	
Materials and Methods	Applicant's version adopted	
Results and discussion	Applicant's version adopted	
Conclusion	Applicant's version adopted	
Reliability	2	
Acceptability	acceptable	
Remarks	none	
	COMMENTS FROM ...	
Date		
Materials and Methods		
Results and discussion		
Conclusion		
Reliability		
Acceptability		
Remarks		

Table A6.6.4- 3: Chromosome analyse of bone marrow cells of rats after dosing with Alphacypermethrin, Cyclophoshamide (positive control) or corn oil (vehicle control).

	Vehicle Control		2 mg/kg bw		4 mg/kg bw		8 mg/kg bw		25 mg/kg bw CPM		40 mg/kg bw CPM	
No. of animals	7	7	6	6	6	6	6	6	7	7	7	7
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
No. of cells analysed	1221	1251	1167	1200	1142	1060	1027	1019	1089	960	744	788
	% cells showing											
Polyploidy[1]	0.1					0.1		0.1				
Chromatid aberrations												
Single	1.1	1.8	0.9	0.8	0.5	0.9	2.2	1.7	6.0	8.9	5.0	9.9
Multiple		0.1			0.1	0.1	0.3	0.1	3.3	7.7	5.2	8.6
Chromosome aberrations												
Single	0.4	0.2	0.3	0.2	0.4	0.3	0.7	0.1	0.5	0.7	0.1	0.6
Multiple							0.1		0.1			0.1
Chromatid + chromosome aberrations	0.1						0.1		0.7	0.8	0.8	1.1
Severe damage[2]									2.3	5.0	6.5	4.7
	Frequency per cell											
Chromatid												
Gaps[3]	0.017	0.014	0.004	0.003	0.003	0.009	0.020	0.012	0.051	0.099	0.043	0.091
Breaks[4]	0.005	0.006	0.004	0.004	0.004	0.002	0.009	0.007	0.082	0.171	0.093	0.171
Exchanges[5]					0.001				0.049	0.078	0.082	0.102
Chromosome												
Breaks[6]	0.005	0.002	0.003	0.002	0.004	0.003	0.010	0.001	0.016	0.017	0.011	0.022
Exchanges[7]										0.001		
Mitotic Index	1.8	1.6	2.3	2.9	2.1	2.2	0.9	2.3	1.2	1.0	0.7	0.6

[1] Includes endoreduplication

[2] Damage so severe that differentiation of aberrations was not possible

[3] Gaps and / or isogaps

[4] Breaks and / or single fragments and / or deletions

[5] Exchange figures including single rings

[6] Acentric fragments

[7] Dicentrics and / or translocations and / or rings

Section A6.6.5 **Genotoxicity *in vivo***
Annex Point IIA 6.6.5 **– ssDNA damage measurement by alkaline elution**

Official use only

1 REFERENCE

1.1	**Reference**	**A6.6.5/01:** [redacted] (1982) Studies on the effect of WL 85871 on the integrity of rat liver DNA *in vivo*. [redacted], Report no. SBGR.81.225, January 1982 (unpublished). (BASF RDI No.: AL-435-004)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	No
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No The basic principle of the study is comparable to method B.39 (2000/32/EC), but differs with respect to exposure time (6 hours instead of 12 to 16 hours after dosing) and the detection method used was liquid scintillation counting instead of autoradiography as proposed in the guideline.
2.2	**GLP**	No
2.3	**Deviations**	Not applicable

3 MATERIALS AND METHODS

3.1	**Test material**	WL 85871, Alphacypermethrin, as given in section A2.
3.1.1	Lot/Batch number	OCD/7; ST81/002
3.1.2	Specification	Not stated
3.1.2.1	Description	Not stated
3.1.2.2	Purity	94.9 %
3.1.2.3	Stability	The test substance was determined to be stable over the period of the experiment.
3.1.2.4	Maximum tolerable dose	Not stated
3.2	**Test Animals**	
3.2.1	Species	Rats

Section A6.6.5 **Genotoxicity *in vivo***
Annex Point IIA 6.6.5 **– ssDNA damage measurement by alkaline elution**

3.2.2	Strain	Wistar
3.2.3	Source	Rodent Breeding Unit of the Shell Toxicology Laboratory, Tunstall, GB
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	Age: 6 to 8 weeks Body weight: males: 350 – 470g; females: 200 – 250g
3.2.6	Number of animals per group	2 males and 2 females
3.2.7	Control animals	Yes 2 males and 2 females
3.3	**Administration/ Exposure**	
3.3.1	Number of application	Single
3.3.2	Interval between applications	Not applicable
3.3.3	Postexposure period	6 h after treatment
3.3.4	Type	Oral by gavage
3.3.5	Dose	40 mg/ kg bw
3.3.6	Vehicle	DMSO
3.3.7	Concentration in vehicle	20% w/v
3.3.8	Total volume applied	Not stated
3.3.9	Controls	Negative control: DMSO Positive control: Methyl methanesulphonate (MMS)
3.4	**Examinations**	
3.4.1	Clinical signs	Not examined
3.4.2	Tissue	Liver from rats previously hepatectomised (partly) and treated with ^{3}H-thymidine: Weight of tissue: 2 g Time points: 6 h after treatment with test compound Type of cells: primary rat hepatocytes Parameters: DNA single strand damage by alkaline elution analysis
3.5	**Further remarks**	None

4 RESULTS

4.1	**Clinical signs**	Not stated

Section A6.6.5 **Genotoxicity *in vivo***
Annex Point IIA 6.6.5 **– ssDNA damage measurement by alkaline elution**

4.2	**Haematology / tissue examination**	Not stated
4.3	**Genotoxicity**	Negative Alphacypermethrin induced no measurable DNA single-strand damage when administered to Wistar rats at a single oral dose of 40 mg/kg bw for an exposure period of 6 hours. Methyl methanesulfonate induced single-strand damage in male and female rat liver DNA in vivo when administered by the oral route at a dose of 350 mg/kg bw.
4.4	**Other**	None

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The ability of Alphacypermethrin to induce DNA single-strand damage was investigated in rat liver cells *ex-vivo* by the alkaline elution assay. The method used corresponds to the principles of method B.39 (2000/32/EC) with some differences.
5.2	**Results and discussion**	The results of this study indicate that neither Alphacypermethrin nor its *in situ* generated metabolites have any effect upon the integrity of rat liver DNA *in vivo*, under the conditions of this experiment.
5.3	**Conclusion**	
5.3.1	Reliability	2
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	March, 2009
Materials and Methods	Applicant's version adopted
Results and discussion	Applicant's version adopted
Conclusion	Applicant's version adopted
Reliability	2
Acceptability	acceptable
Remarks	none
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.6.6 Investigation of germ cell effects

Annex Point IIA 6.6

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible** [ ] **Scientifically unjustified** [X]	
Limited exposure [ ]	**Other justification** [ ]	
Detailed justification:	The assessment of germ cell effects is not considered to be required, for the following reasons: (1) There was no evidence of any mutagenic potential of Alphacypermethrin in the lower tiers of the genotoxicity screening, i.e.: - A6.6.1 (in a bacterial system), - A6.6.2 (in an in-vitro cytogenicity test), - A6.6.3 (in an in-vitro gene mutation assay). (2) Further, Alphacypermethrin also failed to elicit any genotoxic response in an *in-vivo* chromosomal aberration test in mouse bone marrow cells (A6.6.4) and in rat liver cells (A6.6.5). Accordingly, further *in vivo* genotoxicity testing in germ cells is not required.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	March, 2009	
Evaluation of applicant's justification	Applicant's version adopted	
Conclusion	Applicant's version adopted	
Remarks	none	
	COMMENTS FROM ...	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A6.6.7 Further genotoxicity tests

Annex Point IIA 6.6

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible** [ ] **Scientifically unjustified** [X]	
Limited exposure [ ]	**Other justification** [ ]	
Detailed justification:	Performance of a second *in vivo* genotoxicity study is not considered to be required, for the following reasons: (1) There was no evidence of any mutagenic potential of Alphacypermethrin in the lower tiers of the genotoxicity screening, i.e.: - A6.6.1 (in a bacterial system), - A6.6.2 (in an *in-vitro* cytogenicity test), - A6.6.3 (in an *in-vitro* gene mutation assay). (2) Further, Alphacypermethrin also failed to elicit any genotoxic response in an *in-vivo* chromosomal aberration test in mouse bone marrow cells (A6.6.4) and in rat liver cells (A6.6.5). According to chapter 2 of the TNsG on common core data requirements, this data requirement for further genotoxicity testing normally refers only to the case where metabolites of concern are formed in mammals. However, in the case of Alphacypermethrin, it has been demonstrated that only the parent compound itself is of concern, in view of its relatively rapid biotransformation by cleavage of the ester bond. Therefore, any such further *in vivo* genotoxicity testing is not required.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	March, 2009	
Evaluation of applicant's justification	Applicant's version adopted	
Conclusion	Applicant's version adopted	
Remarks	none	
	COMMENTS FROM ...	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A6.7 **Chronic toxicity and carcinogenicity study in rats**
Annex Point IIA 6.7

Official use only

1 REFERENCE

1.1	**Reference**	**Cross-reference to A6.5/01:** [redacted] (1978) 2 year feeding study of WL43467 in rats. [redacted], Report no. TLGR.78.189 (unpublished). BASF RDI No.:CY-427-001 **Cross-reference to A6.5/02:** [redacted] (1979) Corrigendum and addendum I: 2 year feeding study of WL43467 in rats. [redacted] Report no. TLGR.78.189, February 1979, (unpublished). BASF RDI No.:CY-427-002 **Cross-reference to A6.5/03:** [redacted], (1981) Corrigendum and addendum II: 2 year feeding study of WL43467 in rats. [redacted] Report no. TLGR.78.189, February 1981, (unpublished). BASF RDI No.:CY-427-003 **Cross-reference to A6.5/04:** [redacted] (1985) Corrigendum and addendum III: 2 year feeding study of WL43467 in rats. [redacted] Report no. TLGR.78.189, February 1985, (unpublished). BASF RDI No.:CY-427-004
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	No
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

Section A6.7 **Chronic toxicity and carcinogenicity study in rats**
Annex Point IIA 6.7

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No The conduct of the study was consistent to EU method B.30 (88/303/EC) in all important aspects, with the following exceptions: less animals were used (24 males and 24 females were treated instead of 50 animals per sex, the satellite groups contained 6 or 12 animals per sex instead of 20 animals per sex); adrenals and ovaries were not weighed; no urinalysis; no determination of albumin concentration and blood glucose; no gamma glutamyl transpeptidase and no ornithine decarboxylase were measured.
2.2	**GLP**	No At the time of the study conduct, GLP was not compulsory. However, the study was conducted in accordance with the principles of GLP.
2.3	**Deviations**	Not applicable

3 MATERIALS AND METHODS

3.1	**Test material**	Cypermethrin (WL 43467)
3.1.1	Lot/Batch number	30
3.1.2	Specification	Not specified
3.1.3	Purity	98%
3.1.4	Description	Not stated
3.1.5	Stability	No information available
3.2	**Test animals**	
3.2.1	Species	Rat
3.2.2	Strain	Wistar, specific pathogens free (SPF)
3.2.3	Source	Shell Toxicology Laboratory, Tunstall, GB
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	Age: 5 weeks Mean body weight ranges: 139–141 g (male) 118–121 g (females)
3.2.6	Number of animals per group	In total 48 males and 48 females per group (interim sacrifices month 6: 6 males and 6 females; month 12: 6 males and 6 females; month 18: 12 males and 12 females)
3.2.7	Control animals	In total 96 males and 96 females (interim sacrifices month 6: 12 males and 12 females; month 12: 12 males and 12 females; month 18: 24 males and 24 females)

Section A6.7 **Chronic toxicity and carcinogenicity study in rats**
Annex Point IIA 6.7

3.3	**Administration/ Exposure**	
3.3.1	Duration of treatment	24 months (interim sacrifices: 6, 12 and 18 months)
3.3.2	Frequency of exposure	Continuously
3.3.3	Post-exposure period	None
3.3.4	Type	Dietary The test substance was mixed to the food matrix dissolve in acetone, which was subsequently evaporated.
3.3.5	Concentration	1, 10, 100 and 1000 ppm
3.3.6	Vehicle	Diet
3.3.7	Concentration in diet	Stability and concentrations in the diets were within acceptable limits throughout the study.
3.3.8	Total volume applied	Diet *ad libitum*
3.3.9	Controls	Control diet with vehicle
3.4	**Examinations**	
3.4.1	Observations	Yes (daily)
	Clinical signs	Yes (daily)
	Mortality	Yes (daily)
3.4.2	Body weight	Yes (weekly for the first 13 weeks, at week 15 and thereafter at 4 weekly intervals)
3.4.3	Food consumption	Yes (weekly for the first 13 weeks, at week 16 and thereafter at 4 weekly intervals)
3.4.4	Water consumption	No
3.4.5	Haematology	Yes Number of animals: each surviving animal Time points: end of study Parameters: haemoglobin, red blood cell counts, total white blood cells counts, differential white blood cell counts, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, prothrombin time, kaolin-cephalin clotting time.
3.4.6	Clinical chemistry	Yes Number of animals: each surviving animal Time points: end of study Parameters: protein, urea, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, sodium, potassium, chloride.
3.4.7	Urinalysis	No

Section A6.7 **Chronic toxicity and carcinogenicity study in rats**
Annex Point IIA 6.7

3.5	**Sacrifice and pathology**	
3.5.1	Organ weights	Yes Organs: liver, kidneys, testes, spleen, brain and heart.
3.5.2	Gross and histopathology	Yes Macroscopic observations: all animals which died and all surviving animals. Histopathological examination: brain, heart, liver, spleen, kidneys, testes, ovaries, stomach, pancreas, mesenteric lymph nodes, prostate or uterus, thyroid/parathyroid with oesophagus and trachea, thymus, eye and lachrymal glands, lungs, pituitary, adrenals, small intestine, large intestine, salivary glands, urinary bladder, sciatic nerves. Examinations were performed on organs and tissues from control rats, rats fed 100 and 1000 ppm Cypermethrin, rats that died earlier in these groups and any other macroscopic lesion in any tissue.
3.5.3	Other examinations	Yes Numeric incidence and classification of primary neoplasms. Examinations were performed on organs and tissues from control rats, rats fed 100 and 1000 ppm Cypermethrin, rats that died earlier in these groups.
3.5.4	Statistics	Body and organ weights: covariance analysis. Clinical chemistry and haematology: analysis of variance. Statistical significance: Williams' t-test or Dunnett's test as appropriate. Tumour data: actuarial analysis (Peto, R. 1974)
3.6	**Further remarks**	None

4 RESULTS

4.1	**Observations**	
4.1.1	Clinical signs	The general health and behaviour of treated and control rats were similar throughout the study.
4.1.2	Mortality	The survival after 2 years of males and female rats of the control and treated groups was similar. Results are presented in Table A6.7-1.
4.2	**Body weight**	Male and female rats treated with Cypermethrin at 1000 ppm had reduced body weights throughout the study when compared to controls. Reductions were not always statistically significant with no significant differences occurring in female body weights after week 76. Results are presented in Table A6.7-1.

Section A6.7 Chronic toxicity and carcinogenicity study in rats

Annex Point IIA 6.7

4.3	**Food consumption and compound intake**	During the first 13 weeks, small, sometimes statistically significant reductions in food consumption were seen in the 1000 ppm group males and females. Thereafter, only minor fluctuations in food intake were observed. Results are presented in Table A6.7-1.
4.4	**Blood analysis**	
4.4.1	Haematology	In various parameters, minor statistically significant fluctuations were seen in the interim and in the two years groups, but all findings were considered to be of no toxicological significance.
4.4.2	Clinical chemistry	No treatment-related changes were observed. Plasma alkaline phosphatase values in males, exposed to 10, 100 and 1000 ppm (2 years) were reduced compared to controls. However, no correlation was noted between exposure level and the magnitude of the change over a 100-fold range, and no evidence of any compound-related pathological changes was seen. Thus, the finding was not considered to be of toxicological evidence. Blood urea values were increased in male rats in the 1000 ppm group sacrificed after 2 years and were marginally increased in high dose females after 1 year. This finding was not accompanied by any changes in the incidence or severity of nephrosis during or at the end of the study. It was regarded as difficult to clearly define a relationship between these findings and exposure to Cypermethrin. Results are presented in Table A6.7-1.
4.4.3	Urinalysis	No
4.5	**Sacrifice and pathology**	
4.5.1	Organ weights	No organ weight changes of toxicological significance were seen that could be attributed to treatment with Cypermethrin. There were significant increases in either absolute or relative organ weights at 1000 ppm for testes (males, 18 months), liver (males, 6 months), heart (males, 6 months), kidney (males, 12 and 18 months; females, 6 months). However, no consistent patterns were seen, no effects were found after two years and no correlating histopathological or clinical chemistry changes were observed. Therefore, these findings were considered not relevant.
4.5.2	Gross and histopathology	No significant treatment-related macroscopic or histological changes were observed in rats fed Cypermethrin at dietary concentrations of 1 to 1000 ppm for up to two years. Sciatic nerves appeared similar at necropsy in all treatment groups and histopathological evaluations did not show any consistent, treatment-related evidence of degeneration.
4.6	**Other**	Statistical analysis revealed no evidence of increased risk of treatment-related tumour development following dietary exposure to Cypermethrin for up to two years.

Section A6.7 **Chronic toxicity and carcinogenicity study in rats**
Annex Point IIA 6.7

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The chronic toxicity and carcinogenicity of Cypermethrin (WL 43467) was tested in 24 Wistar rats per sex fed the test substance at dietary concentrations of 1, 10, 100, and 1000 ppm. A control group of 48 animals per sex received untreated diet. Groups of rats were scheduled for necropsy after 6 months (6 per sex treated, 12 per sex control), 12 months (6 per sex treated, 12 per sex control), 18 months (12 per sex treated, 24 per sex control) and 2 years (24 per sex treated, 48 per sex control) of treatment. Although not a guideline study, the method used was consistent to EU method B.33 (88/303/EC) in all important aspects, with the following exceptions: less tested animals were used (24 males and 24 females were treated instead of 50 animals per sex, the satellite groups contained 6 or 12 animals per sex instead of 20 dosed animals per sex); adrenals and ovaries were not weighed; no urinalysis; no determination of albumin concentration and blood glucose; no gamma glutamyl transpeptidase and no ornithine decarboxylase were measured.
5.2	**Results and discussion**	Male and female rats exposed to 1000 ppm Cypermethrin had reduced body weights throughout the study. The trend was consistent but not always statistically significant. No significant differences in survival were seen between treatment and control groups. There were no treatment-related effects on haematology, clinical chemistry and organ weights. Macroscopic and histopathological examination did not indicate any biologically significant lesions in any organ or other effects that were considered to be related to treatment. A wide spectrum of tumours and degenerative and other lesions were seen at post-mortem and microscopically. None appeared to be compound-related. Based on decreased body weights for both sexes the NOAEL was determined to be 100 ppm.
5.3	**Conclusion**	
5.3.1	LO(A)EL	1000 ppm
5.3.2	NO(A)EL (chronic effects)	100 ppm, corresponding to 5 mg/kg bw/d (applying a factor of 0.05 for adult rats (GDCh, BUA Grundsatzpapiere, August 1992) for the conversion from "ppm in feed" to "mg/kg bw/d")
5.3.3	NO(A)EL (oncogenic effects)	1000 ppm, corresponding to 50 mg/kg bw/d
5.3.4	Other	
5.3.5	Reliability	2
5.3.6	Deficiencies	No

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	April, 2007
Materials and methods	Applicant's version adopted
Results and discussion	Applicant's version adopted
Conclusion	Applicant's version adopted
Reliability	1
Acceptability	acceptable
Remarks	none
	COMMENTS FROM ...
Date	
Materials and methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.7- 1: Results of the 2-year feeding study in rats.

Parameter	Control		1 ppm		10 ppm		100 ppm		1000 ppm		Dose-response +/–	
	m	f	m	f	m	f	m	f	m	f	m	f
No of animals examined												
6 months	12	12	6	6	6	6	6	6	6	6		
12 months	12	12	6	6	6	6	6	6	6	6		
18 months	24	24	12	12	12	12	12	12	12	12		
2 years	48	48	24	24	24	24	24	24	24	24		
Survival rate [%]												
2 years	67	42	46	33	54	38	71	42	71	50	–	–
Body weight [g]												
Week 1	184	160	184	160	185	160	184	159	166**	153**	–	–
Week 13	462	287	462	281	468	286	448*	286	435**[1]	264**	–	–
Week 75	569	403	582	402	582	393	565	419	534**	375**	–	–
Week 103[a]	552	412	560	384	560	413	524	419	515*	398	–	–
Food intake [g/rat]												
Week 1	135	122	134	121	137	122	133	124	106**	103**	–	–
Week 13	141	132	145	123	139	132	138	131	134*[1]	120**	–	–
Week 103 [a]	112	95	97	78	102	83	108	96	105	86	–	–
Clinical chemistry												
Urea (1 year) [mmol/L]	7.0	9.0	6.7	8.8	6.8	8.9	7.5	8.2	7.5	9.7*		+
Urea (2 years) [mmol/L]	7.8	8.7	7.4	6.7	8.1	6.1	11.3	6.9	12.3**[2]	7.0	+	
AP (2 years) [IU]	72	37	63	32	53*	35	59**	36	56**	40	–	

* significantly greater or less than control mean ($p<0.05$)

** significantly greater or less than control mean ($p<0.01$)

1) No. of observations = 21

2) No. of observations = 16, excludes one outlying value, male 80

a) measured one week early

Section A6.7 **Carcinogenicity test in mice**
Annex Point IIA 6.7

Official use only

1 REFERENCE

1.1	**Reference**	**Cross-reference to A6.5/05:** [redacted] (1996) Alphacypermethrin: Oncogenicity study by dietary administration to CD 1 mice. Report no. 95/SHL010/0596, May 20, 1996 (unpublished). BASF RDI No.: AL-428-002. **Cross-reference to A6.5/06:** [redacted] (1999) Alphacypermethrin: Oncogenicity study by dietary administration to CD 1 mice. First amendment to Report no. 95/SHL010/0596, November 4, 1999 (unpublished). BASF RDI No.: AL-428-003.
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	No
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes OECD guideline 451 (1981) The conduct of the study was consistent to EU method B.32 (88/303/EC) in all important aspects.
2.2	**GLP**	Yes
2.3	**Deviations**	None

3 MATERIALS AND METHODS

3.1	**Test material**	Alphacypermethrin
3.1.1	Lot/Batch number	02156
3.1.2	Specification	As given in Section A2
3.1.3	Purity	95.4%
3.1.4	Description	Aggregative off-white powder
3.1.5	Stability	The test substance was stable during the duration of the study.
3.2	**Test animals**	
3.2.1	Species	Mouse

Section A6.7 Carcinogenicity test in mice

Annex Point IIA 6.7

3.2.2	Strain	CD-1	
3.2.3	Source	Charles River Limited, Margate, Kent, England	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	Age:	30–37 days
		Body weight mean:	27.1 g (males) 22.5 g (females)
3.2.6	Number of animals per group	72 male and 72 female animals (interim phase: 20 animals per sex; terminal phase: 52 animals per sex)	
3.2.7	Control animals	72 males and 72 females (interim phase: 20 animals per sex; terminal phase: 52 animals per sex)	
3.3	**Administration/ Exposure**		
3.3.1	Duration of treatment	78 weeks (interim phase: 52 weeks)	
3.3.2	Frequency of exposure	Continuously	
3.3.3	Post-exposure period	None	
3.3.4	Type	Dietary	
3.3.5	Concentration	30, 100 and 300 ppm.	
3.3.6	Vehicle	Diet	
3.3.7	Concentration in diet	Stability in the diet was confirmed prior to study initiation. Diet sample analysis showed acceptable concentrations (94.7 ± 2.6, 95.3 ± 3.0 and 95.9 ± 3.4 % for 30, 100 and 300 ppm, respectively) and homogeneity.	
3.3.8	Total volume applied	Diet *ad libitum*	
3.3.9	Controls	Control diet	
3.4	**Examinations**		
3.4.1	Observations	Yes (at least twice daily; more detailed examination weekly)	
	Clinical signs	Yes (at least twice daily; more detailed examination weekly)	
	Mortality	Yes (at least twice daily)	
3.4.2	Body weight	Yes (before treatment, weekly for the first 14 weeks of treatment and once every two weeks thereafter and before necropsy)	
3.4.3	Food consumption	Yes (mean weekly consumption was recorded for each cage)	
3.4.4	Food conversion efficiency	Yes (weekly food conversion efficiency was calculated for the first 14 weeks of treatment)	
3.4.5	Water consumption	Yes, (daily by visual appraisal, no quantitative measurements)	
3.4.6	Ophthalmoscopic examination	No	

Section A6.7 Carcinogenicity test in mice

Annex Point IIA 6.7

3.4.7	Haematology	Yes Number of animals: each surviving animal of the high dose and control groups and animals sacrificed prematurely Time points: after week 50 and 77 Parameters: differential white blood cell counts
3.5	**Sacrifice and pathology**	
3.5.1	Organ weights	Yes Organs: adrenals, brain, heart, kidneys, liver, lungs with main stem bronchi, spleen, testes, uterus with cervix
3.5.2	Gross and histopathology	Yes Macroscopic observations: Animals of all dose groups were examined by detailed inspection of external features and orifices, the neck and associated tissues and the cranial, thoracic, abdominal and pelvic cavities and viscera. External and cut surfaces of organs were examined as appropriate. Histopathology: adrenals, aorta, brain, caecum, colon, duodenum, epididymides, eye and optic nerve, femoral bone and stifle joint, gall bladder, heart, ileum, jejunum, kidneys, liver, lungs with mainstem bronchi, lymph nodes (mandibular, mesenteric), mammary gland (caudal), oesophagus, ovaries, pancreas, pituitary, prostate, rectum, salivary gland (submandibular), sciatic nerve, seminal vesicles, skeletal muscle (thigh), skin, spinal cord, spleen, sternum and marrow, stomach (keratinised, glandular), testes, thymus, thyroid with parathyroids, trachea, urinary bladder, uterus with cervix, vagina. Histopathological evaluation was performed on animals of all dose groups. All abnormal organs and tissues found at necropsy were also taken for histological examination.
3.5.3	Other examinations	None
3.5.4	Statistics	Mortality: Cox's test, Tarone's test, Kaplan-Meier method. Haematology: Mann-Whitney test. Organ weights, food consumption and body weight changes: Bartlett's test, Behrens-Fisher test, Dunnett's test as approriate. Pathology/histopathology: Fisher's Exact test.
3.6	**Further remarks**	

Section A6.7 **Carcinogenicity test in mice**
Annex Point IIA 6.7

4 RESULTS

4.1	**Observations**	
4.1.1	Clinical signs	In general, clinical signs of reactions to treatment were found in males of the 300 ppm group and comprised of thin build, ungroomed coats, hair loss and encrustations on the skin. Additionally, males at 100 ppm showed a slightly higher incidence of ungroomed coats in comparison to control. Isolated incidences of hunched posture were also observed in males at 300 ppm. Females were generally unaffected by treatment. However, a slightly increased incidence of over-activity was seen at 300 ppm. The group distribution, location, multiplicity and time of onset of the few palpable swellings was unaffected by treatment. Results are presented in Table A6.7- 3.
4.1.2	Mortality	A total of 104 males and 61 females were killed or died during the study with an even distribution amongst the groups. Treatment of CD-1 mice with Alphacypermethrin did not adversely affect the survival of treated animals; indeed survival amongst females receiving 300 ppm was higher than that seen in controls. Results are presented in Table A6.7- 3.
4.2	**Body weight**	Throughout the treatment period markedly lower body weight gains were seen in males and females receiving 300 ppm resulting in overall lower body weight gains of 26% and 24% than those of controls, respectively. A 13% lower body weight gain was observed in males of the 100 ppm group. Results are presented in Table A6.7- 3.
4.3	**Food consumption and compound intake**	A marginal but non-significant group mean increase in food consumption was recorded for males at 300 ppm which was most apparent between weeks 14 and 26 of treatment. During the first 14 weeks of treatment the overall food conversion efficiency of males at 100 ppm and males and females at 300 ppm Alphacypermethrin was lower than that of the controls. The mean achieved dose levels were 3.0, 10.6 and 35.2 mg/kg bw/d for males and 3.5, 11.5 and 37.7 mg/kg bw/d for females treated with 30, 100 and 300 ppm Alphacypermethrin in the diet, respectively. Results are presented in Table A6.7- 3.
4.4	**Blood analysis**	
4.4.1	Haematology	No treatment related changes were seen in blood smears evaluated after 50 or 77 weeks of treatment and in prematurely sacrificed animals.

Section A6.7 **Carcinogenicity test in mice**
Annex Point IIA 6.7

4.5	**Sacrifice and pathology**	
4.5.1	Organ weights	No inter-group differences in absolute and relative organ weights which were considered to be related to treatment with Alphacypermethrin were noted. A few statistically significant changes in high dose animals were attributed to the marked effect on terminal body weights.
4.5.2	Gross and histopathology	There were no macroscopic changes in animals killed after 52 and 78 weeks of treatment which were considered to be related to treatment. No treatment-related neoplastic or non-neoplastic findings were observed during histopathological examination. A few statistically significant findings were not attributed to treatment but considered to have arisen by chance.
4.6	**Other**	None

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Groups of 72 male and 72 female CD-1 mice received diets containing 0, 30, 100 and 300 ppm of Alphacypermethrin. 20 males and 20 females of each group were sacrificed for interim examination, after 52 weeks, the remainder were sacrificed after 78 weeks of treatment. The study was conducted according to OECD guideline 451 (1981).
5.2	**Results and discussion**	Administration of Alphacypermethrin to CD-1 mice for 78 weeks was associated with a number of non-specific effects. Treatment of animals with 300 ppm Alphacypermethrin resulted in poor growth performance and changes in appearance. In males, this was evident by a higher incidence of thin build during the in-life phase. Signs of reaction to treatment were generally confined to males receiving 300 ppm and included ungroomed coats, hair loss and surface encrustations on the skin and were probably related to the irritant properties of the test material. Other signs of reaction included occasional hunched posture in males and over-activity in females receiving 300 ppm. Overall weight gains of males and females in the 300 ppm group were 26 and 24 % lower than those of the controls and were associated with a reduced efficiency of food conversion. This is considered indicative of a non-specific toxicity rather than an influence of food intake. A similar, less marked effect on weight gain and food conversion efficiency was noted for males receiving 100 ppm. No treatment-related effects on haematology or organ weight changes were seen, and macroscopic and histopathological examinations revealed no changes attributable to treatment with the test substance. There was no evidence that Alphacypermethrin had any oncogenic potential at concentrations up to 300 ppm. Based on the poor growth performance and changes in appearance the NOAEL was determined to be 30 ppm.

Section A6.7 Carcinogenicity test in mice

Annex Point IIA 6.7

5.3	**Conclusion**	
5.3.1	LO(A)EL	100 ppm, corresponding to 10.8 mg/kg bw/d for male and 11.7 mg/kg bw/d for female mice. (conversion from "ppm in feed" to "mg/kg bw/d" calculated according to: LO(A)EL (ppm) x group mean food consumption / group mean body weight)
5.3.2	NO(A)EL	30 ppm, corresponding to 3.0 mg/kg bw/d for male and 3.5 mg/kg bw/d for female mice. (conversion from "ppm in feed" to "mg/kg bw/d" calculated according to: NO(A)EL (ppm) x group mean food consumption / group mean body weight)
5.3.3	Other	
5.3.4	Reliability	1
5.3.5	Deficiencies	No

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Date	April, 2009
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Conclusion	Applicant's version adopted
Reliability	1
Acceptability	acceptable
Remarks	none
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Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.7- 2: Results of the 78-week feeding study in CD1-mice.

Parameter	Control		30 ppm		100 ppm		300 ppm		Dose-response +/–	
	m	f	m	f	m	f	m	f	m	f
No of animals examined										
Terminal phase – 78 weeks	52	52	52	52	52	52	52	52		
Interim phase – 52 weeks	20	20	20	20	20	20	20	20		
Mortality										
Terminal phase	22	21	22	11	21	20	26	7		
Interim phase	3	0	4	1	2	1	4	0		
Clinical signs										
Thin build[1]	3	18	4	18	8	21	19	25	+	+
Ungroomed coat[1]	9	4	7	1	18	3	55	5	+	
Hunched posture[1]	8	14	7	10	7	11	26	8	+	–
Over-activity[1]	0	5	1	6	1	4	2	12		+
Encrustations[1]	35	9	35	3	44	4	50	3	+	–
Hair loss (ventral)[2]	16/65	1/69	10/66	1/72	19/63	0/65	30/65	0/71	+	
Hair loss (dorsal)[2]	16/65	3/69	9/66	1/72	17/63	0/65	35/65	3/71	+	
Body weight gain [g]										
Weeks 0–26	27.3	17.3	26.7	13.8**	23.3**	15.6	20.7**	12.7**	–	–
Weeks 0–78	27.8	23.3	27.1	21.5	24.3	20.7	20.5**	17.8*	–	–
Food intake [g]										
Week 1–78	2795	2528	2802	2355	2817	2361	2922	2389	–	–
Food conversion efficiency [%][3]	4.6	2.7	4.5	2.4	3.7	2.8	3.2	2.2	–	–

* significantly different from control mean ($p<0.05$)

** significantly different from control mean ($p<0.01$)

1) weeks 1–80 (total no. of animals affected)

2) month 12 (maximum monthly incidence; no. of animals affected / no. of alive animals)

3) weeks 1–14

Section A6.8.1 Teratogenicity test in the rabbit

Annex Point IIA 6.8.1

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1 REFERENCE

1.1	**References**	**A6.8.1/01:** [redacted] (1994) Alphacypermethrin – Oral (gavage) rabbit developmental toxicity (teratogenicity) study. [redacted], Report No. SLN/4/93, March 07, 1994 (unpublished). (BASF RDI No.: AL-432-004)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	No
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes US EPA 83-3
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	As given in Section A2
3.1.1	Lot/Batch number	ST 91/243
3.1.2	Specification	As given in Section A2
3.1.3	Purity	95.6%
3.1.4	Description	White powder
3.1.5	Stability	Not stated
3.2	**Test animals**	
3.2.1	Species	Rabbit
3.2.2	Strain	New Zealand White
3.2.3	Source	Interfauna UK Ltd., Huntingdon, UK
3.2.4	Sex	Female
3.2.5	Age/weight at study initiation	Age: approx. 4 months Body weight: 3.0–4.0 kg

Section A6.8.1 Teratogenicity test in the rabbit

Annex Point IIA 6.8.1

3.2.6	Number of animals per group	16 females
3.2.7	Control animals	16 females
3.2.8	Mating period	Not specified in the report. The day of mating was termed day 0 of pregnancy.
3.3	**Administration/ Exposure**	
3.3.1	Duration of exposure	Day 7–19 post mating
3.3.2	Post-exposure period	9 days (sacrifice on day 28 of gestation)
3.3.3	Type	Oral by gavage
3.3.4	Concentration	3, 15, 30 mg/kg bw/day
3.3.5	Vehicle	Corn oil
3.3.6	Concentration in vehicle	Stability for at least 4 hours and homogeneity were established prior to study initiation of the main study. Analysis of formulation samples (first day of dosing and towards the end of the dosing period) gave results between 92 and 101% of nominal concentrations.
3.3.7	Total volume applied	2 mL/kg bw
3.3.8	Controls	Vehicle (corn oil)
3.4	**Examinations**	
3.4.1	Body weight	Yes (on day 3, 7 to 19, 22, 25 and 28 of pregnancy; day 0 body weights were delivered the supplier)
3.4.2	Food consumption	Yes (every two days from day 3 to 27, thereafter every one day from day 27 to 28 of pregnancy)
3.4.3	Clinical signs	Yes (daily between 4 to 7 hours after dosing)
3.4.4	Examination of uterine content	Pregnancy status, gravid uterine weight, number of corpora lutea, number and distribution of implantation sites (early/late resorptions)
3.4.5	Examination of foetuses	Number of live or dead foetuses, foetal bodyweight, sex ratio, external, visceral, head and skeletal abnormalities (all live foetuses)
3.5	**Further remarks**	None

Section A6.8.1 **Teratogenicity test in the rabbit**
Annex Point IIA 6.8.1

4 RESULTS

4.1 Maternal toxic effects

Two control females, three females treated at 15 mg/kg bw/d and two females treated at 30 mg/kg bw/d were sacrificed non-scheduled between day 15 and 22 of pregnancy due to low food consumption and severe body weight loss. None of these non-scheduled sacrifices were considered treatment-related.

No treatment-related clinical signs were observed. Reduced faecal output was seen in all groups and related to the vehicle (corn oil).

One mid-dose female aborted on the scheduled day of necropsy, which was not considered treatment-related. This female had shown weight fluctuations and low food consumption from the onset of dosing and throughout pregnancy. A pale liver and distended stomach and intestines were observed upon necropsy.

Body weight reductions were observed in all treatment groups and the vehicle control during the initial third (Days 7–10) of the dosing period to a similar extent. In the high dose group, a more marked reduction of body weights was observed towards the end of the dosing period. Although not statistically significant, these findings were considered treatment-related, even though there was evidence of recovery following cessation of treatment.

The inter-group differences in mean food consumption generally mirrored the changes in mean body weights. At the onset of the dosing period, food consumption amounted to approx. 30–40% of pre-dose values. During the middle and latter stages, food consumption amounted to approx. 50% of pre-dose values in the control, low and mid dose groups. At 30 mg/kg bw/d food consumption amounted only to approx. 40% of pre-dose values, which was considered treatment-related even though there was evidence of recovery following cessation of treatment for all groups.

The results are summarised in Table A6.8.1- 1.

No treatment-related abnormalities were observed upon necropsy apart from dehydration. No adverse effects on gravid uterus weights, mean number of corpora lutea, numbers of implantations and live foetuses or on pre- or post-implantation loss were observed at any dose level.

Section A6.8.1 **Teratogenicity test in the rabbit**
Annex Point IIA 6.8.1

4.2	**Teratogenic/ embryotoxic effects**	No treatment-related effects on foetal weights and sex ratio were observed. There were no treatment-related effects on the nature or incidence of major or minor external, visceral or skeletal abnormalities or on the incidences of foetuses with variants of development. The number and mean percentages of foetuses with abnormalities were 9 (7.3%), 3 (2.7%), 4 (4.8%) and 1 (1.2%) in the control and groups treated at 3, 15 and 30 mg/kg bw/d, respectively.
4.3	**Other effects**	None

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The effects of Alphacypermethrin on the pregnancy and embryonic or foetal development of the rabbit was investigated at 0 (control), 3, 15 and 30 mg/kg bw/day administered in corn oil from day 7 to day 19 post mating. The study was conducted according to US EPA 83-3. The conduct of the study was consistent in all important aspects to OECD 414 (2001) and EC method B.31 (2004/73/EC), with the exception that less than 20 female animals with implantation sites were investigated, and that the test substance was administered solely during the period of organogenesis.
5.2	**Results and discussion**	There was no maternal or developmental toxicity associated with oral administration at 3 or 15 mg/kg bw/d. Administration via gavage of 30 mg/kg bw/d of Alphacypermethrin to pregnant rabbits during foetal organogenesis caused reduced maternal food consumption and body weights during the latter stages of the dosing period. There was no evidence of embryo or foetal lethality, growth retardation or teratogenicity at any tested dose level.
5.3	**Conclusion**	
5.3.1	LO(A)EL maternal toxic effects	30 mg/kg bw/day
5.3.2	NO(A)EL maternal toxic effects	15 mg/kg bw/day
5.3.3	LO(A)EL embryotoxic/ teratogenic effects	Not applicable
5.3.4	NO(A)EL embryotoxic/ teratogenic effects	30 mg/kg bw/day (highest tested dose level)
5.3.5	Reliability	1
5.3.6	Deficiencies	No

Evaluation by Competent Authorities	
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Date	April, 2009
Materials and methods	Applicant's version adopted
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Conclusion	Applicant's version adopted
Reliability	1
Acceptability	acceptable
Remarks	none
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Remarks	

Table A6.8.1- 1: Maternal effects.

Parameter	**Control**	**3 mg/kg/d**	**15 mg/kg/d**	**30 mg/kg/d**	**Dose response** +/–
Number of dams examined	14	16	11	14	
Mortality of dams [%]	2	0	3	2	
Abortions	0	0	1	0	
Body weight gain day 7–19, [kg] [mean ± SD]	0.07 ± 0.13	0.06 ± 0.16	0.08 ± 0.14	-0.03 ± 0.22	
Body weight gain day 19–28, [kg] [mean ± SD]	0.21 ± 0.06	0.25 ± 0.13	0.28 ± 0.16	0.32 ± 0.07**	
Food consumption, day 7–11, [g/rat/day], [mean ± SD]	47 ± 41	44 ± 29	65 ± 40	48 ± 24	
Food consumption, day 11–15, [g/rat/day], [mean ± SD]	68 ± 34	85 ± 46	79 ± 31	59 ± 28	
Food consumption, day 15–19, [g/rat/day], [mean ± SD]	68 ± 30	71 ± 42	78 ± 43	58 ± 44	
Necropsy findings in dams dead before end of test	none	n.a.	dehydration of the gastro-intestinal contents or body fat		n.a.

n.a.: not applicable
** significantly different from the control group, $p < 0.01$ (Student's t-test)

Section A6.8.1 **Teratogenicity test in the rat**
Annex Point IIA 6.8.1

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1 REFERENCE

1.1	**References**	**A6.8.1/02:** [redacted] (1994) Alphacypermethrin – Oral (gavage) rat developmental toxicity (teratogenicity) study. [redacted], Report No. SLN/2/92, March 07, 1994 (unpublished). (BASF RDI No.: AL-432-002)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	No
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes US EPA 83-3
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	As given in Section A2
3.1.1	Lot/Batch number	ST 91/243
3.1.2	Specification	As given in Section A2
3.1.3	Purity	95.6%
3.1.4	Description	White powder
3.1.5	Stability	Not stated
3.2	**Test animals**	
3.2.1	Species	Rat
3.2.2	Strain	Sprague-Dawley (OFA (SD) IOPS-Caw strain)
3.2.3	Source	Iffa Credo Ltd., Belgium
3.2.4	Sex	Female
3.2.5	Age/weight at study initiation	Age: approx. 9–12 weeks (at mating) Body weight: approx. 225–305g (at mating)

Section A6.8.1 Teratogenicity test in the rat

Annex Point IIA 6.8.1

3.2.6	Number of animals per group	24 females
3.2.7	Control animals	24 females
3.2.8	Mating period	Two to three consecutive days (2 F : 1 M or 1 F : 1 M); day 0 of pregnancy was the day of observation of a sperm positive smear.
3.3	**Administration/ Exposure**	
3.3.1	Duration of exposure	Day 6–15 of gestation
3.3.2	Post-exposure period	5 days (sacrifice on day 20 of gestation)
3.3.3	Type	Oral, by gavage
3.3.4	Concentration	3, 9, 18/15, 15 mg/kg bw/day The dose level was 18 mg/kg bw/d from day 6 to 9 of pregnancy, and 15 mg/kg bw/d from day 10 to 15 of pregnancy. This group was added ten days later than the other groups.
3.3.5	Vehicle	Corn oil
3.3.6	Concentration in vehicle	Stability for at least 4 hours and homogeneity were established prior to study initiation of the main study. Analysis of formulation samples (first day of dosing and towards the end of the dosing period) gave mean recoveries within 11% of nominal concentrations.
3.3.7	Total volume applied	4 mL/kg bw
3.3.8	Controls	Vehicle (corn oil)
3.4	**Examinations**	
3.4.1	Body weight	Yes (on day 0, 6 to 15, and 20 of pregnancy)
3.4.2	Food consumption	Yes (on day 0 to 6, 6 to 9, 9 to 12, 12 to 15, and 15 to 20 of pregnancy)
3.4.3	Clinical signs	Yes (daily immediately and between 4 to 7 hours after dosing)
3.4.4	Examination of uterine content	Pregnancy status, gravid uterine weight, number of corpora lutea, number and distribution of implantation sites (early/late resorptions).
3.4.5	Examination of foetuses	Number of live or dead foetuses, foetal bodyweight, sex ratio, external abnormalities (all live animals), visceral and skeletal abnormalities (one half of the foetuses), combined sectioning/dissection (remaining foetuses).
3.5	**Further remarks**	None

Section A6.8.1 **Teratogenicity test in the rat**
Annex Point IIA 6.8.1

4 RESULTS

4.1	**Maternal toxic effects**	None of the animals died or were sacrificed prematurely. Clinical signs observed in females initially treated at 18 mg/kg bw/d included unsteady gait, piloerection, limb splay, hypersensitivity to sound, excess salivation and hunched posture following administration of the second to fourth dose. Four females were particularly affected and showed intermittent rolling and hypersensitivity to touch, three of these were later observed to be prostrate. Clinical signs diminished 2 days after reduction of the dose level. In animals treated only with 15 mg/kg bw/d, limb splay, unsteady gait, hypersensitivity to sound and touch and piloerection were noted following 4–8 administrations. Clinical signs observed in this group were less severe than those observed at 18/15 mg/kg bw/d. Treatment-related effects on body weight and food consumption were observed at 15 and 18/15 mg/kg bw/d during the dosing period. There was a compensatory increase in food consumption and body weight gain following cessation of treatment in the 15 mg/kg bw/d group. No treatment-related clinical signs, effects on food consumption or body weight gain were observed at 3 and 9 mg/kg bw/d. The results are summarised in Table A6.8.1-2. No treatment-related abnormalities were observed upon necropsy. No adverse effects on gravid uterus weights, mean number of corpora lutea, numbers of implantations and live foetuses or on pre- and post-implantation loss were observed at any dose level.
4.2	**Teratogenic/ embryotoxic effects**	No treatment-related effects on sex ratio were observed. Treatment-related slight reduction of mean foetal weights were observed at 15 and 18/15 mg/kg bw/d when compared to the control group. This reduction was statistically significant in the female foetuses and for the overall foetal weights at the 15 mg/kg bw/day dose level only. At 3 and 9 mg/kg bw/d, no treatment-related effects on foetal weights were observed. There were no treatment-related effects on the nature or incidence of major or minor external, visceral or skeletal abnormalities or on the incidences of foetuses with variants of development. However, the mean number of foetuses with minor skeletal abnormalities was significantly increased at 3 and 18/15 mg/kg bw/day when compared to the control. These increased values were mainly due to higher incidences of foetuses with retarded ossification of sternebrae 2 and to a lesser extent, sternebrae 3 and 4 and also with bilobed, bipartite or misaligned sternebrae. However, the values were within the expected background range and were not dose-related. In addition, no other indications of retardation of skeletal ossification were observed. Thus, they were considered to be unrelated to treatment. The results are summarised in Table A6.8.1- 4 and Table A6.8.1- 5.
4.3	**Other effects**	None

Section A6.8.1 **Teratogenicity test in the rat**
Annex Point IIA 6.8.1

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The effects of Alphacypermethrin on the pregnancy and embryonic or foetal development of the rat were investigated at 0 (control), 3, 9, 18/15 and 15 mg/kg bw/day administered in corn oil by gavage from day 6 to day 15 post mating. The study was performed according to US EPA 83-3. The conduct of the study is consistent in all important aspects to OECD 414 (2001) and EC method B.31 (2004/73/EC), with the exception that the test substance was administered solely during the period of organogenesis.
5.2	**Results and discussion**	Administration of Alphacypermethrin via gavage at 15 or 18 mg/kg bw/d to pregnant rats during foetal organogenesis elicited maternal toxicity characterised by changes in clinical conditions and reduction in food consumption and body weight gain during the dosing period. Further, a slight reduction in foetal weights was observed at these dose levels most probably being a consequence of the observed maternal toxicity. However, there was no other evidence of embryo- or foetotoxicity or teratogenicity at any tested dose level.
5.3	**Conclusion**	
5.3.1	LO(A)EL maternal toxic effects	15 mg/kg bw/day
5.3.2	NO(A)EL maternal toxic effects	9 mg/kg bw/day
5.3.3	LO(A)EL embryotoxic/ teratogenic effects	15 mg/kg bw/day
5.3.4	NO(A)EL embryotoxic/ teratogenic effects	9 mg/kg bw/day
5.3.5	Reliability	1
5.3.6	Deficiencies	No

Evaluation by Competent Authorities	
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Date	April, 2009
Materials and methods	Applicant's version adopted
Results and discussion	Applicant's version adopted
Conclusion	Applicant's version adopted
Reliability	1
Acceptability	acceptable
Remarks	none
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Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.8.1- 2: Maternal effects.

Parameter	Control	3 mg/kg/d	9 mg/kg/d	18/15 mg/kg/d	15 mg/kg/d	Dose response +/–
Number of dams examined	24	24	24	24	23	
Clinical findings during application of test substance	–	–	–	yes[1]	yes[2]	+
Body weight gain, day 6–15, [g] [mean ± SD]	71 ± 10	69 ± 9	68 ± 9	56 ± 10***	40 ± 15***	+
Body weight gain, day 15–20, [g] [mean ± SD]	87 ± 10	81 ± 12	83 ± 11	80 ± 12	98 ± 18**	+
Food consumption, day 6–9, [g/rat/day], [mean ± SD]	26.7 ± 3.0	27.1 ± 3.0	26.2 ± 2.5	22.3 ± 2.9***	20.5 ± 4.0**	+
Food consumption, day 9–12, [g/rat/day], [mean ± SD]	28.7 ± 3.4	27.0 ± 5.8	27.6 ± 2.0	23.7 ± 2.5***	24.3 ± 4.7***	+
Food consumption, day 12–15, [g/rat/day], [mean ± SD]	30.0 ± 4.0	30.8 ± 4.0 (23)	29.3 ± 2.9	28.0 ± 2.2*	26.9 ± 4.0**	+

* significantly different from the control, $p < 0.05$, Student's t-test

** significantly different from the control, $p < 0.01$, Student's t-test

*** significantly different from the control, $p < 0.001$, Student's t-test

1 unsteady gait, piloerection, limb splay, hypersensitivity to sound and touch, excess salivation, hunched posture

[2] limb splay, unsteady gait, hypersensitivity to sound and touch, piloerection

Table A6.8.1- 3: Litter response (Caesarean section data).

Parameter	Control	3 mg/kg/d	9 mg/kg/d	18/15 mg/kg/d	15 mg/kg/d	Dose response +/–
Foetal weight, males [g] [mean ± SD]	4.10 ± 0.27	4.06 ± 0.41	4.14 ± 0.23	3.96 ± 0.24	3.93 ± 0.24	
Foetal weight, females [g] [mean ± SD]	3.91 ± 0.25	3.82 ± 0.39	3.93 ± 0.23 (23)	3.74 ± 0.34	3.71 ± 0.24*	+
Foetal weight, all [g]	4.01 ± 0.26	3.92 ± 0.39	4.06 ± 0.23	3.84 ± 0.32	3.82 ± 0.22*	+

* significantly different from the control, $p < 0.05$, Student's t-test

Table A6.8.1- 4: Examination of the foetuses.

Parameter	**Control**	**3 mg/kg/d**	**9 mg/kg/d**	**18/15 mg/kg/d**	**15 mg/kg/d**	**Dose response +/–**
Skeletal examination						
Total number of foetuses (litters) examined	187 (24)	177 (24)	190 (24)	179 (24)	186 (23)	
Number with minor abnormalities only	8 (5)	20 (12)	8 (5)	20 (11)	12 (10)	
Mean %	4.2	13.1*	4.0	12.8*	6.3	
Number with major abnormalities	0(0)	0 (0)	0 (0)	0 (0)	1 (1)	
Mean %	0.0	0.0	0.0	0.0	0.4	

* significantly different from control, $p < 0.05$, Dunn's multiple comparison test

Section A6.8.1 Teratogenicity

Annex Point IIA 6.8.1 – Supportive data –

The following references are considered to contain additional information concerning doses chosen for the teratogenicity tests summarised above and are thus presented in an abbreviated format (adopted from the PPP-dossier) as supportive data:

Developmental Toxicity in Rabbits – Dose Range Finding Study

Reference: **A6.8.1/03**

[redacted] (1994) Alphacypermethrin – Oral (gavage) rabbit developmental toxicity dose ranging study. [redacted] Report No. SLN/3/92, March 07, 1994 (unpublished). (BASF RDI No.: AL-432-003).

Guidelines: Not applicable (range finding study only)

GLP: Yes

Methods:

This preliminary study in New Zealand white rabbits consisted of two parts. The first part was a study in non-mated females to establish the maximum tolerated dose (MTD). The second part was a study in mated females to investigate the effect of the test article on the pregnant rabbit and offspring in utero in order to select dose levels for a subsequent developmental toxicity study.

MTD Phase: A group of five non-mated, sexually mature, female New Zealand White (NZW) rabbits was dosed, once daily, by oral gavage with solutions of alphacypermethrin in corn oil for twenty five days within a thirty day period. The dose levels increased as follows; 10 mg/kg b.w./day for days 1 to 5, 15 mg/kg b.w./day for days 6 to 10, 25 mg/kg b.w./day for days 12 to 16, 35 mg/kg b.w./day for days 19 to 23, and 40 mg/kg b.w./day for days 26 to 30. On intervening days, the animals were not dosed. Once the MTD was established, a second group of five non-mated females was dosed, once daily, orally by gavage at 35 mg/kg b.w./day for seven consecutive days.

Mated Phase: Groups of 5 timed-mated NZW rabbits were dosed, once daily, by oral gavage from days 7 to 19 of pregnancy, inclusive, at dose levels of 5, 15, 25, and 30 mg/kg b.w./day alphacypermethrin in corn oil. A group dosed with the vehicle only served as controls. On day 28 of pregnancy, animals were sacrificed by caesarean section. The fetuses were weighed, sexed and subjected to external examination.

Findings:

Increasing Dose MTD Phase: During the first two days of dosing at 10 mg/kg b.w./day, there were slight reductions in body weight and food consumption values. The animals recovered, however, and there were no further signs of treatment at 10, 15, or 25 mg/kg b.w./day. At 35 mg/kg b.w./day, one animal appeared ataxic and languid on the first day of dosing, and there was a trend toward a slight decrease in body weight, food consumption and feces production during the five days of treatment at this dosage. These animals recovered in the two off-dose days. At 40 mg/kg b.w./day, the animals showed slight fluctuations in body weight and food consumption, similar to the 35 mg/kg b.w./day dose group. The MTD was considered to have been reached at 35 mg/kg b.w./day. There were no treatment-related abnormalities at necropsy.

Fixed dose MTD Phase: The protocol specified that the animals would be dosed for eight days, however, dosing was discontinued after seven days because of the significant toxicity associated with the treatment. There were no unscheduled deaths, but two animals were sacrificed prematurely (one on day 6 and one on day 7) due to negligible food consumption and marked body weight loss. All animals showed body weight loss and reduced fecal output after one or two doses, which persisted throughout dosing; however, on day 9, two days after cessation of dosing, two of the three surviving females showed a slight body weight gain. There was a marked decrease in food consumption during dosing, as well, with appetite recovery observed for all animals after cessation of dosing. There was a lack of food found in the stomachs of those females sacrificed prematurely, however there were no abnormalities at necropsy that were considered to be related to treatment.

Mated phase: Two females (one in each group at 15 and 25 mg/kg b.w./day) were sacrificed prematurely because of low food consumption values and body weight loss. Clinical signs were limited to a reduction in the

amount of feces for control and treated animals. Animals in the control, 25 and 30 mg/kg/day groups exhibited a mean body weight loss at the onset of dosing. This may have been the result of the low tolerance of the pregnant rabbit to administration of corn oil (vehicle). The weight gains of the animals dosed at 5 and 15 mg/kg/day were unaffected by treatment. There was a trend toward further reductions in mean body weights during the latter third of the treatment period for animals dosed with 25 and 30 mg/kg/day only. After the conclusion of the dosing period, all affected animals showed a compensatory increase in mean body weight. Food consumption was similarly reduced during the dosing period in animals which exhibited weight loss; a slight reduction in food consumption was also noted during the treatment period for animals dosed at 5 and 15 mg/kg/day. Mean food consumption for all groups returned to expected levels at the cessation of treatment.

There were no treatment-related abnormalities at maternal necropsy and no apparent adverse effects of treatment on mean numbers of corpora lutea, implantations, live fetuses, or pre- or post- implantation losses. There was no effect of treatment on fetal weight, sex ratio, or the incidence of fetuses with external abnormalities.

Dose levels established for the main study were 3, 15 and 30 mg/kg b.w./day. The highest dose level was selected as a dose expected to elicit minimal maternal toxicity; higher doses were not recommended in light of the toxicity observed at 35 mg/kg/day in the preliminary MTD study. The lowest level (3 mg/kg/day) was selected as a probable "no effect" level with respect to maternal toxicity.

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	April, 2009
Materials and methods	Applicant's version adopted
Results and discussion	Applicant's version adopted
Conclusion	Applicant's version adopted
Reliability	2 (no guideline)
Acceptability	acceptable
Remarks	none
	COMMENTS FROM ...
Date	
Materials and methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	