Developmental Toxicity in Rats – Dose Range Finding Study

Reference: **A6.8.1/04**

[redacted] (1994) Alphacypermethrin – Oral (gavage) rat developmental toxicity dose ranging study. [redacted], Report No. SLN/1/92, March 07, 1994 (unpublished). (BASF RDI No.: AL-432-001).

Guidelines: Not applicable (range finding study only)

GLP: Yes

Methods:

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

MTD Phase: Groups of three non-mated, sexually mature, female Sprague-Dawley rats were dosed, once daily, orally by gavage, with solutions of alphacypermethrin in corn oil vehicle for five consecutive days. Dose levels of 15, 18, 20, 30 and 50 mg/kg were employed.

Mated Phase: Groups of five timed-mated Sprague-Dawley rats were dosed, once daily, by oral gavage, from days 6 through 15 of pregnancy at dose levels of 3, 9, 15, and 18 mg/kg b.w./day alphacypermethrin in corn oil. A group dosed with the vehicle only served as controls. On day 20 of pregnancy a necropsy was performed. The fetuses were weighed, sexed and subjected to external examination.

Findings:

MTD Phase: Treatment at levels of 20, 30 and 50 mg/kg b.w./day was associated with severe changes in clinical condition, including convulsions, ataxia, hypersensitivity to touch and sound, spasms, piloerection, limb splay and hunched posture that were typical pyrethroid-associated effects. The severity of the effects was dose-related. Dosing was discontinued prematurely, after the second treatment for these groups, and all animals in the 50 and 30 mg/kg b.w./day groups and one animal in the 20 mg/kg b.w./day group were sacrificed. In the 18 mg/kg b.w./day group, there were similar clinical signs of toxicity as described above, as well as weight loss. On day 5, prior to dosing, one female displaying severe clinical signs of toxicity was sacrificed. The others were dosed as scheduled. In the 15 mg/kg b.w./day group, all three females were dosed for five consecutive days; animals in this group exhibited slight weight loss, and two had observations of piloerection and/or hunched posture. None of the surviving females showed treatment-associated abnormalities at necropsy. The MTD was determined to be 18 mg/kg b.w./day.

Mated phase: Clinical signs of treatment at 18 mg/kg b.w./day included hind limb splay and unsteady gait. One female treated at 15 mg/kg day also exhibited similar signs. One female in the 9 mg/kg b.w./day group in poor clinical condition, was sacrificed on day 16; the condition of this animal was determined not to be treatment associated as similar signs were not seen at higher dose levels. No treatment-related clinical changes were noted in other animals in the 9 mg/kg b.w./day group or at lower dose levels.

Mean body weight gain in the 18 mg/kg b.w./day group was significantly reduced in comparison to the control group over the 6-15 day dosing period. Body weight gains in the groups treated at 9 and 15 mg/kg b.w./day were lower during the first half of the dosing period, but the differences were not statistically significant and a compensatory increase was observed in the 15-20 day interval. Body weight gain in the group treated at 3 mg/kg b.w./day was similar to that of the control group throughout the dosing period. Similar to body weight gains, mean food consumption values in the 18 mg/kg b.w./day group were lower than that of the control group over the entire dosing period, and were significantly lower during days 6-12. Food consumption values in the 15 mg/kg b.w./day group were slightly, but not significantly lower between days 6 and 9 only. At the lower dose levels, food consumption values were similar to that of the control group throughout the dosing period.

There were no treatment-related abnormalities at maternal necropsy. There were no significant intergroup differences in the mean numbers of corpora lutea, implantations or live fetuses. Pre- and post-implantation losses were increased, as compared to the controls, for all treatment groups and post-implantation losses were statistically significantly increased for the 9 mg/kg b.w./day group. These effects were believed, however, to be related to unusually low pre- and post-implantation losses in the control animals and were not considered to be toxicologically significant. There were no effects of treatment on fetal weights or sex ratios.

Dose levels established for the main study were between 3 and 18 mg/kg b.w./day. The highest dose level was selected as a dose expected to elicit minimal maternal toxicity, and the lowest level 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	

Section A6.8.2 Three generation reproduction study

Annex Point IIA 6.8.2

Official use only

1 REFERENCE

1.1	**References**	**A6.8.2/01:** [redacted] (1978) Toxicity studies on the insecticide WL 43467 – A three generation reproduction study in rats. [redacted], Report no. TLGR.0188.78 (unpublished). (BASF RDI No.: CY-430-001) **A6.8.2/02:** [redacted] (1979) Corrigendum I: Toxicity studies on the insecticide WL 43467 – A three generation reproduction study in rats. [redacted], Report no. TLGR.0188.78, February 1979, (unpublished). (BASF RDI No.: CY-430-002) **A6.8.2/03:** [redacted] (1979) 2nd Corrigendum: Toxicity studies on the insecticide WL 43467 – A three generation reproduction study in rats. [redacted], Report no. TLGR.0188.78, September 1979, (unpublished). (BASF RDI No.: CY-430-003) **A6.8.2/04:** [redacted] (1981) Addendum and corrigendum III: Toxicity studies on the insecticide WL 43467 – A three generation reproduction study in rats. [redacted], Report no. TLGR.0188.78, January 1981, (unpublished). (BASF RDI No.: CY-430-004) **A6.8.2/05:** [redacted] (1985) Fourth addendum/corrigendum: Toxicity studies on the insecticide WL 43467 – A three generation reproduction study in rats. [redacted], Report no. TLGR.0188.78, April 1985, (unpublished). (BASF RDI No.: CY-430-005a)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF AG
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 A6.8.2 **Three generation reproduction study**
Annex Point IIA 6.8.2

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No The conduct of the study was consistent in all important aspects to OECD 416 (1983), with exception that the test procedure was adapted to three generations, that males of the P- generation were dosed for less than 10 weeks prior to mating, and that food consumption and body weight were only recorded during the pre-mating period.
2.2	**GLP**	No GLP was not compulsory at the time when the study was performed. However, the study was audited by a quality assurance unit.
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
3.2.3	Source	Shell Toxicology Laboratory (Tunstall)
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	Age: approx. 5 weeks Body weight: 31–74 g (males) 34–69 g (females)
3.2.6	Number of animals per group	30 males and 30 females (P generation, but less in the other generations due to mating not resulting in pregnancy, single sex litters, one or no pups surviving to weaning) For details refer to Table A6.8.2- 1.
3.2.7	Mating	One female : one male
3.2.8	Duration of mating	Not specified
3.2.9	Deviations from standard protocol	Second mating of parents, F1b and F2b animals. Litters were not standardised (culled) in any generation.

Section A6.8.2 Three generation reproduction study

Annex Point IIA 6.8.2

3.2.10	Control animals	Yes
3.3	**Administration/ Exposure**	
3.3.1	Animal assignment to dosage groups	See Table A6.8.2- 1
3.3.2	Duration of exposure before mating	5 weeks
3.3.3	Duration of exposure in general (P, F1, F2, males, females)	Continuously Pups of F1a, F2a, F3a, and F3b litters and pups of the second litters not selected for mating were killed at weaning (day 21). Parental animals of each generation were killed at weaning of each second litter.
3.3.4	Type	Dietary
3.3.5	Concentration	10, 100, 500 ppm
3.3.6	Vehicle	In food The test substance was dissolved in 60 ml acetone (1 mL/kg diet) prior to mixing with the diet.
3.3.7	Concentration in diet	Cypermethrin was found stable in experimental diet for the period of usage of one batch of diet.
3.3.8	Total volume applied	Diet *ad libitum*
3.3.9	Controls	Control diet with solvent
3.4	**Examinations**	
3.4.1	Clinical signs	Yes (daily)
3.4.2	Body weight	Yes (weekly during pre-mating periods up to the age of 10 weeks, immediately before mating for F1 and F2)
3.4.3	Food consumption	Yes (weekly during pre-mating periods up to the age of 10 weeks, immediately before mating for F1 and F2)
3.4.4	Oestrus cycle	Not determined
3.4.5	Sperm parameters	Not determined
3.4.6	Offspring	Number of pups born alive/dead Sex of pups alive on day 1 Number and sex of pups dying during lactation (pre-weaned deaths) Number and sex of pups weaned Total litter weights (on days 1, 4, 7, 14, 21) Individual pup weights (on day 21) Presence of gross anomalies
3.4.7	Macroscopic pathology	All parental animals of each generation were subjected to gross pathological examinations.

Section A6.8.2 **Three generation reproduction study**
Annex Point IIA 6.8.2

3.4.8	Organ weights P, F1 and F2	No
3.4.9	Histopathology F2b adults and F3b weanlings	One male pup and one female pup and their parents from ten final (F3b) litters in the control and the high dose group were subjected to histopathology. The following tissues were examined: Brain, heart, liver, spleen, kidneys, testes, ovaries, stomach, pancreas, lymph nodes, prostate, uterus, urinary bladder, thyroid, thymus, parathyroid, eye, lungs, pituitary, adrenals, small intestine, large intestine, oesophagus, salivary glands, sciatic nerve and any other tissues showing macroscopic lesions.
3.4.10	Statistics	Body, litter and pup weights, food intakes: covariance analysis. Statistical significance: Williams' test or Dunnett's test as appropriate. No. of litter, fertility index, no. of litters with dead pups: Fisher's Exact test or its asymptotic equivalent. Litter sizes: Wilcoxon two-sample rank sum test.
3.5	**Further remarks**	None

4 RESULTS

4.1 Effects

4.1.1 Parent animals (P)

Parental animals	General health and behaviour were similar between control and treated groups and there were no significant effects on fertility and reproductive performance. Significantly reduced body weights were observed in high dose females during test week 5 to 7 when compared to the controls. Reduced food intake, believed to be related to non-palatability of the diet, were noted for females at 500 ppm (week 3 to 7) and sporadically for males at 10, 100 and 500 ppm. Reduced food intake observed in females of the high dose group in week 5–7 was associated with adverse body weight effects and was thus considered treatment-related. The results are summarised in Table A6.8.2- 2.
Litter (F1)	The mean litter size based on all litters born was statistically significantly reduced in high dose F1a animals on day 0 (total/alive), and days 1 to 21 (alive). Number of female pups per litter was also statistically significantly lower on day 1 and 21. Other statistically significant effects on litter size were not considered of toxicological relevance. No treatment-related pathological changes were observed. The mean litter weights on day 7, 14 and 21 were significantly reduced in this group (F1a). At weaning on day 21, mean pup weights were significantly reduced for high dose female pups and pups of both sexes of the second litter (F1b) when compared to the control. In this group, mean pup weights were also consistently lower from day 1 to 14 but this was not statistically significant. The results are summarised in Table A6.8.2- 4.

Section A6.8.2 **Three generation reproduction study**
Annex Point IIA 6.8.2

4.1.2	F1 animals	
	Parental animals	General health and behaviour were similar between control and treated groups and there were no significant effects on fertility and reproductive performance. No treatment-related pathological changes were observed. Statistically significantly reduced body weights were observed in high dose males (week 5 to 7) and females (week 3 to 7) when compared to the controls. Reduced food intake, considered treatment-related, was noted for high dose females (week 4–7). The results are summarised in Table A6.8.2- 2.
	Litter (F2)	No adverse effects were observed.
4.1.3	F2 animals	
	Parental animals	General health and behaviour were similar between control and treated groups and there were no significant effects on fertility and reproductive performance. No treatment-related pathological changes were observed. High dose females showed significantly reduced food intake (week 5 and 7) and reduced body weight (week 4 to 7). The results are summarised in Table A6.8.2- 2.
	Litter (F3)	Mean pup weights of F3b males were significantly reduced on day 21 at 10, 100 and 500 ppm. Since reduced mean pup weights were observed for F3b males only and no effects on litter weights were found, these reduced pup weights were regarded as biologically irrelevant. The results are summarised in Table A6.8.2- 4.

Section A6.8.2 **Three generation reproduction study**
Annex Point IIA 6.8.2

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Groups of approx. 30 Wistar rats per sex in each generation received Cypermethrin at dietary concentrations of 0, 10, 100 or 500 ppm throughout the entire experimental period up to weaning of F3 litters (pre-mating, mating, gestation and rearing). Two litters were produced from each parental generation. The conduct of the study was consistent to OECD 416 (1983) in all important aspects, with the exception that the test procedure was extended to three generations, that males of the P-generation were dosed for less than 10 weeks prior to mating, and that food consumption and body weight were only recorded during the pre-mating period.
5.2	**Results and discussion**	No mortality occurred during the study. General health and behaviour of treated animals were comparable to control animals. Significantly reduced body weights and food consumption were observed in males and females of the parental generations at the 500 ppm level prior to mating. Minor reproductive effects were recorded at 500 ppm, comprising lower litter sizes and weights and lower pup weights, mainly for the P-generation. At 10 and 100 ppm, no treatment-related adverse effects on parents or litters at any generation were observed. Upon necropsy, no compound-related gross or microscopic pathological findings were observed.
5.3	**Conclusion**	
5.3.1	LO(A)EL	
	Parent animals (P, F1, F2)	500 ppm corresponding to approx. 50 mg/kg bw/d (*cf.* Table A6.8.2- 3) (reduced food intake and body weight during pre-mating period)
	Reproduction (F1, F2, F3)	500 ppm corresponding to approx. 50 mg/kg bw/d (*cf.* Table A6.8.2- 3) (reduced litter size at birth primarily in the F1a generation, and reduced mean pup weights on day 21 for F1b females and F3b males)
5.3.2	NO(A)EL	
	Parent animals (P, F1, F2)	100 ppm corresponding to approx. 10 mg/kg bw/d (*cf.* Table A6.8.2- 3)
	Reproduction (F1, F2, F3)	100 ppm corresponding to approx. 10 mg/kg bw/d (*cf.* Table A6.8.2- 3)
5.3.3	Reliability	2
5.3.4	Deficiencies	Yes Males of the P-generation were dosed for less than 10 weeks prior to mating.

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 (The conduct of the study was consistent to OECD 416 (1983) in all important aspects, with the exception that the test procedure was extended to three generations, that males of the P-generation were dosed for less than 10 weeks prior to mating, and that food consumption and body weight were only recorded during the pre-mating period.)
Acceptability	acceptable
Remarks	none
	COMMENTS FROM ...
Date	
Materials and methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.8.2- 1: Table for animal assignment for mating.

		Number of animals			
		Control	**10 ppm**	**100 ppm**	**500 ppm**
Parents[1]	Male	30	30	30	30
	Female	30	30	30	30
F1[2]	Male	28	25	28	25
	Female	28	25	28	25
F2[3]	Male	18	20	19	19
	Female	18	20	19	19

1) Pairs remained together and were allowed to produce 2 litters. Litter F1a was killed at weaning (day 21). Weaned second litters (F1b) were randomly selected for mating.

2) Pairs remained together and were allowed to produce 2 litters. Litter F2a was killed at weaning (day 21). Weaned second litters (F2b) were randomly selected for mating

3) Pairs remained together and were allowed to produce 2 litters. Litter F3a and F3b were killed at weaning (day 21).

Note: The terminology used to designate the parental and offspring groups of animals in the study report is different from the currently accepted designators. The report uses "F0, F1,and F2" to describe the original parental and first, second and third generation offspring generations with appropriate subscripts for the first or second litter in that generation. Current terminology employs the descriptors "P0, F1 and F2" to describe the parental generations and F1, F2 and F3, with the added subscript "a" or "b" to describe the offspring. The terminology used here follows the latter scheme and updates that used in the report.

Table A6.8.2- 2: Observed effects on food consumption and body weight during the pre-mating period.

				Control		10 ppm		100 ppm		500 ppm	
Parameter		**Genera-tion**	**Week**	**male**	**female**	**male**	**female**	**male**	**female**	**male**	**Female**
Food intake	Mean [g]	P	3	163.7	140.3	**157.1***	135.5	**158.3***	139.2	**152.3****	**131.3****
			4	192.8	157.2	**181.2****	151.5	**184.6***	152.5	186.9	**147.1****
			5	189.9	147.9	**183.0***	143.3	185.8	143.0	185.0	**137.3****
			6	208.8	155.4	202.3	156.9	204.1	156.1	202.1	**148.6***
			7	212.3	157.2	209.4	157.3	207.6	155.2	**202.7****	**148.2****
		F1	3	188.4	157.2	**173.1****	156.2	182.5	152.2	186.6	153.4
			4	207.7	173.1	207.4	163.8	205.5	**158.5***	205.3	**160.1***
			5	209.2	168.6	208.6	163.7	210.2	163.1	203.9	**158.5***
			6	214.7	166.1	209.7	163.8	211.8	158.2	214.6	**154.4***
			7	214.2	170.3	213.8	167.0	213.5	166.5	213.1	**159.2***
		F2	3	169.4	145.4	173.1	143.3	173.5	151.6	170.6	137.9
			4	194.9	161.8	181.4	149.4	186.8	163.2	199.0	141.0
			5	198.0	159.2	194.1	147.6	192.7	159.5	187.9	**134.8****
			6	203.4	154.1	200.6	147.7	195.5	159.6	199.4	144.1
			7	205.6	162.4	201.4	152.8	196.2	160.6	197.8	**144.9****
Body weight	Mean [g]	P	3	185.7	152.7	184.2	151.5	187.4	154.4	183.3	149.0
			4	239.2	174.6	237.1	174.0	240.6	177.2	236.7	169.4
			5	287.7	201.5	288.6	200.7	291.2	202.3	283.9	**192.1****
			6	328.7	215.8	328.0	216.9	331.4	219.0	321.3	**207.7***
			7	360.5	232.1	360.5	232.6	362.9	234.5	350.8	**222.1***
		F1	3	223.3	168.5	219.9	169.6	221.8	167.2	220.5	**162.0***
			4	280.8	197.3	277.9	195.6	278.6	194.8	272.8	**186.6****
			5	330.3	219.4	324.4	217.8	327.5	218.1	**319.1***	**206.4****
			6	370.6	239.8	364.5	238.0	366.8	238.0	**357.2***	**224.4****
			7	404.6	256.3	395.4	253.2	397.4	254.9	**385.2****	**238.1****
			Prior to mating	477.9	297.8	470.7	289.6	473.6	295.8	467.9	**273.6****
		F2	3	232.7	173.8	224.2	170.0	229.1	173.9	236.7	170.3
			4	291.1	202.0	281.5	196.7	283.3	203.1	284.5	**192.4****
			5	334.1	221.5	325.2	215.8	327.0	225.0	329.0	**210.4***
			6	373.1	239.4	364.6	231.8	362.6	243.2	363.7	**225.2***
			7	400.8	253.6	393.1	246.3	391.3	256.6	389.8	**238.3***
			Prior to mating	483.6	303.1	480.4	293.8	473.6	308.9	479.9	**283.6***

Note: Administration of test diet commenced at week 2.

* statistically significantly different compared to control ($p \leq 0.05$); ** ($p \leq 0.01$)

Table A6.8.2- 3: Test compound intake at relevant dietary concentrations.

		Test compound intake (mg/kg bw/d)*	
		100 ppm	**500 ppm**
Parents	Male	9.9	50.3
	Female	11.2	56.4
F1	Male	9.6	48.9
	Female	11.1	57.2
F2	Male	9.0	44.8
	Female	10.8	51.4

* calculated with weekly mean values (week 2–7);
NOAEL/LOAEL = NOAEC/LOAEC × daily food consumption/body weight

Table A6.8.2- 4: Observed effects on mean **litter size** (based on all litters born).

Parameter	**Day**	**Litters**	**Control**	**10 ppm**	**100 ppm**	**500 ppm**
Mean litter size F1a	0	Total	13.1	11.5	13.8	10.6 **
	0	Alive	12.9	11.4	13.8	10.5**
	1	Alive	12.0	10.7	12.9	10.0**
	4	Alive	11.7	10.2	12.7	9.4**
	7	Alive	11.7	10.1	12.6	9.3**
	14	Alive	11.7	9.9*	12.5	9.2**
	21	Alive	11.7	9.9*	12.4	9.2**
	1	Females	6.3	5.1	6.1	4.6**
	21	Females	6.0	4.7*	5.8	4.3**
Mean litter weight [g]	7	–	168.1	146.7	176.0	137.2*
F1a	14	–	321.7	287.7	330.6	266.5*
	21	–	527.2	466.3	543.8	435.3*
Mean pup weight [g] F1b	21	Females	47.6	47.6	47.1	43.2 *
	21	Total	48.4	48.0	48.1	44.5*
Mean pup weight [g] F3b	21	Males	56.5	51.1 *	50.3 *	51.2 *

* statistically significantly different compared to control ($p \leq 0.05$)

Section A6.8.2 Reproductive toxicity

Annex Point IIA 6.8.2 **– Supportive data –**

The following references are from the open literature or statements to the open literature and were included into the Evaluation based on a decision taken at the Technical meeting in Varese-Italy in March 2013 and are considered to contain additional information concerning reproductive toxicity and are thus presented in an abbreviated format (adopted from the PPP-dossier) as supportive data:

Male fertility study in mice

Reference: **A6.8.2/02**

Al-Hamdani NM, Yajurvedi HN. Cypermethrin reversibly alters sperm count without altering fertility in mice. Ecotoxicol Environ Saf. 2010 Jul;73(5):1092-7.

Guidelines: No

GLP: No

Material and methods (taken from the abstract):

Administration of cypermethrin (CYP), orally by gavage (3 doses: 1.38, 2.76, and 5.52 mg/kg body weight) [as a 10% EC-formulation of cypermethrin of unknown composition], to mice either for 6 (D1) or 12 (D2) weeks.

Findings (taken from the abstract):

[The treatment]... caused a significant reduction in epididymal spermatozoa count and an increase in abnormal spermatozoa count when compared to controls. These counts returned to normal levels 6 weeks after cessation of 1.38 or 2.76 mg/kg body weight (BW) treatment either after D1 or D2. In 5.52 mg/kg BW treated mice the counts returned to normal levels following D1 but not after D2. Mice in all the treatment groups showed normal fertility. Weight of the litter born to mice mated with CYP treated (all three doses) males either in D1 or D2 was significantly lower than controls whereas gestation period and litter size did not significantly vary from controls. This is the first report revealing that CYP as low as 1.38 mg/kg BW adversely affects spermatogenesis and that the effect is reversible up to 2.76 mg/kg BW/kg BW exposure for 3 months. The results further reveal that despite reduction in sperm count and increase in proportion of abnormal spermatozoa, normal fertility is possible. Hence, in reproductive toxicity evaluation of pesticides, fertility test alone is misleading.

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, 2013
Materials and methods	
Results and discussion	
Conclusion	Adverse effects on reproduction were seen at dose levels that induced general toxicity as shown by the decreased body weight gain (nutritional status is known to affect the male rat reproductive system). As such, cypermethrin might have affected the animals indirectly rather than having any specific effect on the reproductive function.
Reliability	3
Acceptability	RMS does not agree with Applicant that the conclusion of this study is that adverse effects on fertility were observed. Only reduction in sperm count was reported.
Remarks	none
	COMMENTS FROM APPLICANT
Date	April, 2013 (taken from the Statement BASF Doc ID 2012/1220476 & 2013/1071382)
Materials and methods	Test substance of unknown composition – only 10 % were Cypermethrin. There are several methodical flaws, like low number of animals, no control group for recovery data, animals kept at higher temperature (27+/- 2 °C) than recommended 18-23°C, which might have resulted in hyperthermia and thereby could lead to adverse effects on spermatogenesis, number of sperm counts are given per epididymidis (cauda) but should be per gram, insufficient information on: age of animals, general toxicity, body weight gain or food consumption, organ weights and histopathology.
Results and discussion	There are several methodical flaws as pointed out which together with the unknown composition of the test substance do not allow drawing conclusions from this study.
Conclusion	The relevance of the results reported in this study is considered questionable
Reliability	3 (not reliable)
Acceptability	
Remarks	none

Male fertility study in rats

Reference:	**A6.8.2/03**
	Elbetieha A, Da'as SI, Khamas W, Darmani H. Evaluation of the toxic potentials of Cypermethrin pesticide on some reproductive and fertility parameters in the male rats. Arch Environ Contam Toxicol. 2001 Nov;41(4):522-8.
Guidelines:	No
GLP:	No

Material and methods (taken from the abstract):

Adult male Sprague-Dawley rats were exposed to tap water containing 0, 8,571, 17,143, or 34,286 ppm cypermethrin for 12 weeks. Based on water consumption per animal per day the rats received 13.15, 18.93, and 39.66 mg cypermethrin, respectively.

Findings (taken from the abstract):

Fertility was significantly reduced in male rats ingesting cypermethrin at a concentration of 13.15 and 18.93 mg in that the number of females impregnated by them was significantly reduced. The number of implantation sites was significantly reduced in females mated with males that had ingested cypermethrin at a concentration of 39.66 mg. A significant reduction in the number of viable fetuses was observed in females impregnated by the exposed males at all three doses of cypermethrin. The body weight gain was significantly lower in the treated males. Ingestion of cypermethrin at a concentration of 18.93 or 39.66 mg per day resulted in a significant increase in the weights of testes and seminal vesicles. Preputial gland weights were increased at all three concentrations of cypermethrin. Epididymal and testicular sperm counts as well as daily sperm production were significantly decreased in exposed males. The serum levels of testosterone, follicle-stimulating hormone and luteinizing hormone were significantly reduced in males exposed to 39.66 mg per day. Ingestion of cypermethrin at 18.93 and 39.66 mg/ animal/day also resulted in a significant decrease in the perimeter and number of cell layers of the seminiferous tubules. The testes of treated animals were infiltrated with congested blood vessels with marked hemorrhage and a significant accumulation of connective tissue surrounding the seminiferous tubules, which contained a large number of immature spermatids. These results clearly demonstrate the adverse effects of cypermethrin pesticide on fertility and reproduction in male rats.

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	May 2013
Materials and methods	Applicant's version adopted
Results and discussion	Applicant's version adopted
Conclusion	Applicant's version adopted
Reliability	3
Acceptability	3
Remarks	
	COMMENTS FROM APPLICANT
Date	April, 2013 (taken from the Statement BASF Doc ID 2012/1220476 & 2013/1071382)
Materials and methods	Test substance is of unknown composition – only 10 % were Cypermethrin. There are several methodological deficiencies: Hormone levels questionable for FSH and LH (kits mentioned are ELISAs specific for human proteohormones), seminal vesicles were stripped of fluid before weighing which is against current guidelines (eg OECD 416); preputial gland weight is not a relevant endpoint (according OECD guidelines), tissues fixed in 10% NBF which should not be used (see OECD 421/422 as morphology is not preserved especially as shrinkage occurs making evaluation of tubule diameter impossible; evaluation not stage aware, cell layers as a lone criterion is not relevant without information on germ cell generations affected. Relevant parameters missing: Prostate weight; no other male genital organs examined histologically; no data on food consumption. No mention of health status of animals, no age is given for females or cycle normality ensured.
Results and discussion	Complete data questionable and highly overdosed indicated by body weight loss
Conclusion	The relevance of the results reported in this study is considered questionable
Reliability	3 (not reliable)
Acceptability	not acceptable
Remarks	none

15d-male adult rat assay

Reference: **A6.8.2/04**

Hu JX, Li YF, Li J, Pan C, He Z, Dong HY, Xu LC. Toxic effects of Cypermethrin on the male reproductive system: with emphasis on the androgen receptor. J Appl Toxicol. 2011 Dec 6. doi: 10.1002/jat.1769.

Guidelines: (Mode of action screening assay within the Endocrine Disruptor Screening Program of the EPA)

GLP: No

Material and methods (taken from the abstract):

The 15-day intact adult male assay was used to investigate the reproductive toxicity of cypermethrin. We also evaluated the contributions of the androgen receptor (AR) to cypermethrin-induced reproductive impairments. Sixty adult male Sprague–Dawley rats were randomly divided into five groups and treated with different doses of cypermethrin (0, 6.25, 12.5, 25 and 50mgkg_1 per day) by oral gavage for 15 days. After the rats were sacrificed, the testes, epididymides, seminal vesicles and prostates were excised and weighed. One testis was frozen to be used for daily sperm production. Another testis was processed for AR immunohistochemical analysis and electron microscopic observation.

Findings (taken from the abstract):

We found that the weights of prostates were significantly decreased in cypermethrin treatment at doses of 25 and 50mgkg_1 per day. Rats treated with cypermethrin at 50mgkg_1 per day exhibited a significant reduction in testicular daily sperm production. Seminiferous tubule changes were noted, including atrophy and distorted seminiferous tubules, reduction and deformation of spermatogonia, spermatocyte and disordered arrangement of spermatoblasts. Ultrastructural changes were found in cypermethrin- treated groups with disrupted cellular junctions, abnormal morphology of the nucleus, necrosis of spermatogonia spermatocytes and Sertoli cells. To clarify the possible mechanism, AR expression [in testis] and the serum levels of testosterone were assayed. AR levels were significantly reduced in the rats treated with cypermethrin and the serum levels of testosterone were reduced in cypermethrin treatment at a dose of 50mgkg_1 per day. These data suggested that cypermethrin can induce impairments of the structure of seminiferous tubules and spermatogenesis in the male rats. The impairments can be attributed to the reduced AR expression.

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

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	May 2013
Materials and methods	Applicant's version adopted
Results and discussion	Applicant's version adopted
Conclusion	Applicant's version adopted
Reliability	3
Acceptability	3
Remarks	

	COMMENTS FROM APPLICANT
Date	April, 2013 (taken from the Statement BASF Doc ID 2012/1220476 & 2013/1071382)
Materials and methods	Differences to the published 15d male adult rat assay protocol (e.g. number of animals, organ weights). Histology: Fixation of testes was done with Bouin's 10%NBF- not clear if that means a mixture or transfer from one fixative to the other, fixation time is not specified. Immunohistochemistry: Bouin's gives unsatisfactory results for AR staining (Latendresse et al, 2002- no staining in central tubules). What was the nuclear counterstain in AR receptor immunostains- how was staging performed if nuclei are not clearly visible? (Periodic acid Schiff and Hematoxylin (PAS+H) is recommended for staging but not performed here. LH- and FSH radioimmunoassays from Beijing North Institute of Biological Technology, Beijing, China are RIAs for measurement of human FSH and LH hormones.
Results and discussion	Regarding Testosterone levels and sperm counts: Data not comparable to other published studies (Xu et al., 2004). Regarding histology and immunohistochemical analysis of AR: Distortion is too general a term to be meaningful, what happened with different germ cells, staging was apparently done but why then be not specific – were certain populations missing - number of layers is not a good criterion. Again, the specific histological findings associated with decreased testosterone are not found nor their absence discussed. Bouin's fixation as well as formalin leads to shrinkage of tubules and chromatin condensation - artefact of fixation (Latendresse, 2002). Electron microscopic pictures were not convincing. Regarding organ weights: The exposure investigated caused significant lower body weight - the presented absolute organ weights are misleading-it is likely that the relative prostate weight shows no significant difference to control. Cypermethrin is a microsomal enzyme inducer- so liver weight should be rather increased at least relative to body weight. Primary effects on testes, epididymis or seminal vesicles were not seen but reduced prostate weight. Regarding Weight of evidence: There is –if at all- only one organ affected- biological plausibility of an anti-androgenic activity is missing. Hormone level effects are only relevant if clear primary effects are seen.
Conclusion	Effects on testis morphology were unlikely to have been missed in regulatory studies especially in regard to the fact that some of the studies were read by one of the leading experts in male pathology (Creasy DM). Based on methodological deficits and / or flaws in the argumentation in the reported study and based on documentation deficits the results in this paper seem not to be valid. The relevance of the results reported in this study is considered questionable.
Reliability	3 (not reliable)

Acceptability	Not acceptable
Remarks	

21 day postnatal exposure of mice over maternal milk including recovery period

Reference: **A6.8.2/05**

Wang H, Wang SF, Ning H, Ji YL, Zhang C, Zhang Y, Yu T, Ma XH, Zhao XF, Wang Q, Liu P, Meng XH, Xu DX. Maternal Cypermethrin exposure during lactation impairs testicular development and spermatogenesis in male mouse offspring. Environ Toxicol. 2011 Aug; 26(4):382-94.

Guidelines: No

GLP: No

Material and methods (taken from the abstract):

In the present study, we investigated the effects of maternal cypermethrin exposure during lactation on testicular development and spermatogenesis in male offspring. Maternal mice were administered with cypermethrin (25 mg/kg) by gavage daily from postnatal day 0 (PND0) to PND21.

Findings (taken from the abstract):

Results showed that the weight of testes at PND21 was significantly decreased in pups whose mothers were exposed to cypermethrin during lactation. Maternal cypermethrin exposure during lactation markedly decreased the layers of spermatogenic cells, increased the inside diameter of seminiferous tubules, and disturbed the array of spermatogenic cells in testes of pups at PND21. In addition, maternal cypermethrin exposure during lactation markedly reduced mRNA and protein levels of testicular P450scc, a testosterone (T) synthetic enzyme. Correspondingly, the level of serum and testicular T at weaning was significantly decreased in pups whose mothers were exposed to cypermethrin during lactation. Although the expression of testicular T synthetic enzymes and serum and testicular T in adulthood had restored to control level, the decreased testicular weight and histological changes were irreversible. Importantly, the number of spermatozoa was significantly decreased in adult male offspring whose mothers were exposed to cypermethrin during lactation. In conclusion, maternal cypermethrin exposure during lactation permanently impairs testicular development and spermatogenesis in male offspring, whereas cypermethrin-induced endocrine disruption is reversible.

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	May 2013
Materials and methods	Applicant's version adopted
Results and discussion	Applicant's version adopted
Conclusion	Applicant's version adopted
Reliability	3
Acceptability	3
Remarks	
	COMMENTS FROM APPLICANT
Date	April, 2013 (taken from the Statement BASF Doc ID 2012/1220476 & 2013/1071382)
Materials and methods	No adequate description of methods: The number of animals used is not clear. Sperm numbers are not normalized to weight of cauda, which is a methodical flaw which renders the results questionable. Investigation of maternal toxicity is not specified. Furthermore there are several flaws in reporting of the study.
Results and discussion	The description of histopathological findings does not use accepted nomenclature e.g. Creasy 2001 so that the relevance of reported findings (disturbed array of spermatogenic cells) is questionable. Photos for immunohistochemistry do not show any convincing disturbance. Although germ cells start to populate the testis before puberty, day 21 might be too early for testicular evaluation. Hormone measurements for testosterone were performed for day 21 animals thereby is within the period of lowest testosterone levels. The major androgen in the testis at day 21 is androstanediol.
Conclusion	The relevance of the results reported in this study is considered questionable.
Reliability	3 (not reliable)
Acceptability	Not acceptable
Remarks	

Reference: **A6.8.2/06**

[redacted] (2012): Alphacypermethrin (BAS 310 I) - Evaluation of the endocrine disrupting potential of Alphacypermethrin (BASF Doc ID: 2012/1220476 and Amendment: 2013/1071382)

Guidelines: not applicable

GLP: not applicable

Executive Summary and Conclusion:

End of May 2012 the Rapporteur Belgium distributed the consolidated commenting table (dated May, 28th 2012) within the Annex I inclusion process of Alphacypermethrin (PT 18). Therein (Comment No. 134), the German competent authority requested the Rapporteur to consider four explicitly named publications on Cypermethrin (Elbethia et al., 2001, Hu et al., 2011, Al-Hamadani & Yajurvedi, 2010 and Wang et al., 2011) which indicate endocrine effects in male rats and mice.

BASF SE was asked to re-evaluate the regulatory database of Alphacypermethrin in order to evaluate whether Alphacypermethrin can be suspected to interfere with the endocrine system. This has been done with a special focus on male reproductive system and is summarized in **PART A** of this statement.

Furthermore BASF SE reviewed the publications and set them in context with the relevant regulatory toxicity studies. This Assessment was performed by BASF experts ([redacted] for histopathology issues and overall discussion, [redacted] for reproductive toxicity issues and Strauss V. for clinical pathology issues and [redacted] for the regulatory aspects) and is given within this statement under **PART B**.

Based on this assessment BASF SE comes to the following conclusion:

- **All regulatory toxicity studies summarized in Part A demonstrate that Alphacypermethrin and / or Cypermethrin do not reveal hormone mediated toxicity.** Clinical signs of neurotoxicity are common for Alphacypermethrin/Cypermethrin treatment in rats, mice and dogs as well as bodyweight reduction accompanied by liver weight increases. The liver effects are considered as adaptive induction of microsomal enzyme activity. Testes weight changes were only seen in combination with reduced body weight and can, without histopathological correlate, considered to reflect body weight changes. No study revealed any treatment-related histopathological findings in epididymides, seminal vesicles, prostate or testes especially as the 90 day studies in mice (AL-425-006) and rat (AL-425-007) were histopathologically investigated in 1993 and 1994 by the leading expert in male pathology, DM Creasy. In addition there was no evidence for hormonally induced carcinogenicity in life time studies in rats and mice with Cypermethrin and Alphacypermethrin. Fertility studies showed lower litter sizes and weights and lower pup weights at parental toxic doses mainly for the first generation. Histopathological investigations in the F2 generation males and F3B weanlings showed no pathological changes in testes and prostate.
- Although on first glance it looked like the publications would complement the regulatory data base for sperm parameters and testosterone levels, the comprehensive review given in Part B demonstrates that **none of the publications showed convincingly, that Cypermethrin causes testicular dysfunction or hormonal disruption.**
- **Therefore, the summarized database provides no evidence that Cypermethrin or Alphacypermethrin induces endocrine disruption.**
- The lack of any preferential distribution of Alpha-cypermethrin to male (or female) reproductive organs, as verified in detailed tissue distribution studies involving oral dosing, further supports the conclusion that Alphacypermethrin is not an endocrine disrupting chemical.

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	May 2013	
Materials and methods		
Results and discussion		
Conclusion		
Reliability		
Acceptability		
Remarks		
	COMMENTS FROM ...	
Date		
Materials and methods		
Results and discussion		
Conclusion		
Reliability		
Acceptability		
Remarks		

Section A6.9 **Neurotoxicity study**
Annex Point IIA 6.9 **– Acute oral neurotoxicity study in the rat –**

Official use only

1 REFERENCE

1.1	**References**	**A6.9/01:** [redacted] (1993) WL85871 (FASTAC) – An acute oral (gavage) neurotoxicity study in the rat. [redacted], Report No. SBTR.92.027, December 20, 1993 (unpublished). (BASF RDI No.: AL-451-004)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF AG
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 in all important aspects to EPA OPPTS 870.6200 (1998), with exception that the pupillary light reflex, the limb rotation and extensor thrust were not included in the FOB.
2.2	**GLP**	Yes
2.3	**Deviations**	Not applicable

3 MATERIALS AND METHODS

3.1	**Test material**	Alphacypermethrin, FASTAC (WL85871)
3.1.1	Lot/Batch number	02156, drum 1085
3.1.2	Specification	Not specified
3.1.3	Purity	95.6%
3.1.4	Description	Off white powder
3.1.5	Stability	Alphacypermethrin was considered to be stable for the duration of the study based on its physical-chemical characteristics.
3.2	**Reference substance**	Not applicable
3.3	**Test animals**	
3.3.1	Species	Rat
3.3.2	Strain	Crl: CD: BR

Section A6.9 **Neurotoxicity study**
Annex Point IIA 6.9 **– Acute oral neurotoxicity study in the rat –**

3.3.3	Source	Charles River, Manston, Kent, U.K.
3.3.4	Sex	Male and female
3.3.5	Age/weight at study initiation	Age: 44–48 days Body weight: 173.1–183.7 g (males) 171.1–171.9 g (females)

3.3.6 Number of animals per group

Group	Dose [mg/kg]	Definitive study		Additional study	
		M	F	M	F
1	0	10	10	5	5
2	4	10	10	5	5
3	20	10	10	6	5
4	40	10	10	6	5

3.3.7	Control animals	Yes, see 3.3.6
3.4	**Administration/ Exposure**	
3.4.1	Exposure	Single dose
3.4.2	Post-exposure period	14 days
3.4.3	Type	Oral (gavage)
3.4.4	Dose levels	4, 20 and 40 mg Alphacypermethrin/kg
3.4.5	Vehicle	Corn oil
3.4.6	Concentration in vehicle	0.6 % solution in corn oil
3.4.7	Stability in vehicle	The stability of the test substance in the vehicle was confirmed for 4 days. The mean concentration in the 0.6% formulation was 90.2% of nominal.
3.4.8	Total volume applied	0.67–6.67 ml/kg
3.4.9	Controls	Vehicle (corn oil)
3.5	**Examinations**	
3.5.1	Body weight	Prior to dosing and thereafter at weekly intervals.
3.5.2	Clinical signs	Yes (twice daily on week days and once daily on weekends and public holidays)

Section A6.9 **Neurotoxicity study**
Annex Point IIA 6.9 **– Acute oral neurotoxicity study in the rat –**

3.5.3	Clinical assessment of neurotoxicity	Number of animals: all rats of the definitive study. Time points: prior to treatment, 5 hours after dosing, day 7 and 14. Observations: Functional observational battery (FOB): autonomic function (lacrimation, salivation, piloerection , exophthalmos, urination, defecation, palpebral closure), convulsion, tremors, abnormal motor movements, reactivity to general stimuli, arousal or state of alertness, posture, gait abnormalities, sensorimotor response to stimuli, body weight, unusual or abnormal behaviour, activity and righting ability; Fore and hind limb grip strength test; Hind limb landing foot splay test; Motor activity assessment.
3.5.4	Pathology	
3.5.4.1	*Whole body perfusion fixation*	Number of animals: 5 rats per sex from each dose group (definitive study) and all surviving rats in the additional study. Time point: at the end of the study. Histopathological examinations: brain, eyes and optic nerve, femoral and gastrocnemius with sural muscle, sciatic nerve (proximal and distal), sural nerve, spinal cord (cervical, thoracic, lumbar), spinal ganglia (cervical, lumbar) from high dose and control animals. Sciatic nerves from mid dose group animals were examined in addition due to findings in the high dose group.
3.5.4.2	*Immersion fixation rats*	Number of animals: 5 rats per sex from each group (definitive study only). Time point: at the end of the study. Macroscopic examination: external body surface, all orifices, body, cranial cavity, thoracic, abdominal and pelvic cavities with associated organs and tissues and the neck with associated organ and tissues. The following tissues were preserved, but not examined histopathologically: brain, eyes/optic nerve, muscle, nerves, spinal cord and any macroscopic lesions.
3.5.5	Statistic	Fisher's exact test (incidences of histopathology lesions), non parametric trend test or Wilcoxon signed rank test (continuous data), chi-squared test (discrete data).
3.6	**Further remarks**	A range finding test (10, 30, 40, 50 or 60 mg/kg Alphacypermethrin) was carried out for dose level selection.

4 RESULTS

4.1	**Mortality**	There were no deaths at 4 mg/kg. One male each of the 20 mg/kg and 40 mg/kg groups (additional study) were found dead the day after dosing.

Section A6.9 **Neurotoxicity study**
Annex Point IIA 6.9 **– Acute oral neurotoxicity study in the rat –**

4.2	**Clinical signs**	Clinical signs of neurotoxicity observed between 3 and 8 hours after dosing at 20 and 40 mg/kg Alphacypermethrin were similar in both studies. The most common signs in males included abnormal / splayed gait, trashing, prostration, vocalization, piloerection, hunched posture, unkempt appearance, soiled/stained body areas and diarrhoea. In females, similar signs were seen, but at a lower frequency. In addition, there were also isolated cases of twitching, tremors, abasia, hypersensitivity, pale eyes, soft faeces and thinning of the fur. Clinical signs had usually resolved three days after dosing. Results are presented in Table A6.9- 1 and Table A6.9- 2.
4.3	**Body weight**	There were no adverse effects on bodyweight gain at any treatment level. Animals gained weight as expected.
4.4	**Clinical assessment of neurotoxicity**	
4.4.1	Functional observational battery (FOB)	There were no treatment-related changes at the 4 mg/kg dose level. At 5 hours post dosing on day 1, most males at 20 and 40 mg/kg showed gait abnormalities (laterally deviated static limb position, splaying or dragging hind limbs, altered fore and hind limb extension) and clinical signs of increased neurological reactivity (greater scores for posture in home cage, removal from home cage, handling of rats). Effects were more pronounced in males treated at 40 mg/kg; similar effects were seen in females, but to a lesser extend. Most animals had recovered by day 7. Additionally, there were effects on righting reflex (males), fur appearance (males) and arousal (females) at 40 mg/kg and salivation at 20 mg/kg (males). Results are presented in Table A6.9- 3.
4.4.2	Fore and hind limb grip strength test	There was no adverse treatment related effect on fore and hind limb grip strength.
4.4.3	Hind limb landing foot splay test	There were no adverse treatment-related differences in hind limb landing foot splay.
4.4.4	Motor activity assessment	There were no treatment-related differences in motor activity parameters.
4.5	**Pathology**	
4.5.1	Macroscopic findings	There were no treatment-related findings.

Section A6.9 **Neurotoxicity study**
Annex Point IIA 6.9 **– Acute oral neurotoxicity study in the rat –**

4.5.2	Histopathology	There were no adverse microscopic findings at 4 mg/kg Alphacypermethrin. Very slight to slight fibre degeneration in the sciatic nerves was the only finding which appeared to show any relationship to treatment with Alphacypermethrin. The changes were observed more frequently in the proximal than the distal part of the nerves. The findings were seen at all dose levels including control in both studies, but the incidence was statistically significantly increased only in the 20 and 40 mg/kg bw groups of the additional study. Results are presented in Table A6.9- 4 und Table A6.9- 5.
4.6	**Other**	None

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The acute neurotoxicity of Alphacypermethrin was studied in two separate acute studies, each using four groups of either 10 animals of each sex (definitive study) or 5 animals of each sex (additional study). The animals received single oral doses of 0, 4, 20 or 40 mg/kg Alphacypermethrin in corn oil, based on a range-finding test. Animals were observed for 14 days. The study was not a guideline study; however the conduct was consistent to EPA OPPTS 870.6200 (1998) in all important aspects, except that the pupillary light reflex, the limb rotation and extensor thrust were not included in the FOB.

Section A6.9 **Neurotoxicity study**
Annex Point IIA 6.9 **– Acute oral neurotoxicity study in the rat –**

5.2	**Results and discussion**	There were no deaths or adverse clinical or pathological treatment-related effects at the 4 mg/kg dose level. Clinical signs of neurotoxicity (e.g. abnormal / splayed gait, trashing, prostration, vocalization, piloerection, hunched posture, unkempt appearance, soiled / stained body areas and diarrhoea) were seen in male rats dosed with 20 mg/kg and 40 mg/kg Alphacypermethrin, 3 to 8 hours after dosing. In females, similar signs were seen, but at a lower frequency, indicating a reduced susceptibility to Alphacypermethrin. Also in the FOB significant differences from control were seen in most males dosed 20 mg/kg and 40 mg/kg of Alphacypermethrin, 5 hours after dosing. Signs included greater scores for posture in home cage, removal from home cage, handling rat in hand, fur appearance, salivation, limb position, abnormal gait, extension effects and righting reflexes. Females were less affected; at the 40 mg/kg dose level they showed significant difference in arousal. No adverse treatment-related differences were seen in fore and hind limb grip strength, in hind limb landing foot splay and in motor activity parameters. There were no treatment-related macroscopic findings. The only histopathological finding which appeared to show any relationship to treatment was very slight to slight sporadic fibre degeneration in the sciatic nerve, which was more frequent in the proximal part of the nerve. This finding was observed at all levels, including control, but the incidence was statistically significantly increased only in the 20 and 40 mg/kg groups of the additional study. This is a well established effect of pyrethroids on the sciatic nerve which is considered to be a secondary effect of the primary biochemical action on the axon.
5.3	**Conclusion**	
5.3.1	LO(A)EL	20 mg/kg
5.3.2	NO(A)EL	4 mg/kg (based on clinical signs of toxicity and evidence of slight sporadic sciatic nerve degeneration in animals at 20 mg/kg)
5.3.3	Reliability	2
5.3.4	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	In 1.1. Reference : (1994) not (1993)
Results and discussion	Applicant version's adopted
Conclusion	Applicant version's adopted
Reliability	2
Acceptability	acceptable
Remarks	none
	COMMENTS FROM APPLICANT
Date	4 May 2009
Materials and Methods	Signature and release date is 20th December 1993, which we consider as the key criterion.
	COMMENTS FROM RAPPORTEUR MEMBER STATE
Date	May 2009
Materials and Methods	Remarks of applicant accepted

Table A6.9- 1: Clinical observation of rats of the definitive study group.

	Males				Females			
Dose Level [mg/kg]	**0**	**4**	**20**	**40**	**0**	**4**	**20**	**40**
Group size	10	10	10	10	10	10	10	10
Abnormal / splayed gait			7	7			1	1
Trashing			2	2			1	
Prostration			2	2				
Vocalization			1	2			1	3
Piloerection				4			2	
Hunched posture			1					
Unkempt appearance			2	4			2	
Soiled / stained body areas	2		4	7			2	1
Diarrhoea	1		2	3			4	2

Table A6.9- 2: Clinical observation of rats of the additional study group.

	Males				Females			
Dose Level (mg/kg)	**0**	**4**	**20**	**40**	**0**	**4**	**20**	**40**
Group size	5	5	6	6	5	5	5	5
Abnormal / splayed gait			4	4				1
Trashing			1	4			1	1
Prostration			1	3			1	
Vocalization				2			1	
Piloerection			5	5				
Hunched posture			1	2				
Unkempt appearance			1	3			1	
Soiled / stained body areas			2	4			1	1
Diarrhoea				1	1	1	1	1

Table A6.9- 3: Results of functional observational battery (FOB) assessment.

	Males				Females			
Dose Level (mg/kg)	**0**	**4**	**20**	**40**	**0**	**4**	**20**	**40**
Posture in home cage			*	*				
Removal from home cage			*					
Handling in hand			*					
Fur appearance				*				
Salivation			*					
Arousal				*				**
Static limb position				*				
Abnormal gait type				*				
Fore limb extension				*				
Hind limb extension				*				
Righting reflex				***				

* $p \leq 0.05$; statistical significant differences from control in chi-squared or Wilcoxon's test

** $p \leq 0.01$; statistical significant differences from control in chi-squared or Wilcoxon's test

*** $p \leq 0.001$; statistical significant differences from control in chi-squared or Wilcoxon's test

Table A6.9- 4: Incidence of fibre degeneration in sciatic nerves of rats (5 animals per group) of the definitive study.

	Males				Females			
Dose Level (mg/kg)	**0**	**4**	**20**	**40**	**0**	**4**	**20**	**40**
Proximal sciatic nerve								
Very slight degeneration	2	2	1	3	1	1	1	0
Slight degeneration	0	0	0	1	0	0	0	0
Total incidence	2	2	1	4	1	1	1	0
Distal sciatic nerve								
Very slight degeneration	0	3	1	2	0	0	0	0
Total incidence	0	3	1	2	0	0	0	0
Total fibre degeneration (proximal and distal)	2	5	2	4	1	1	1	0

Table A6.9- 5: Incidence of fibre degeneration in sciatic nerves of rats (5 animals per group) of the additional study.

	Males				Females			
Dose Level (mg/kg)	**0**	**4**	**20**	**40**	**0**	**4**	**20**	**40**
Proximal sciatic nerve								
Very slight degeneration	0	0	5**	4*	0	2	2	1
Slight degeneration	1	1	0	1	0	0	2	0
Total incidence	1	1	5*	5*	0	2	4*	1
Distal sciatic nerve								
Very slight degeneration	1	1	2	3	0	0	1	2
Total incidence	1	1	2	3	0	0	1	2
Total fibre degeneration (proximal and distal)	1	2	5*	5*	0	2	4*	3

* $p<0.05$; ** $p<0.01$ significance in a pairwise (Fisher's) test between each treatment and control incidence.

Section A6.9 **Neurotoxicity study**

Annex Point IIA 6.9 **– Neurochemical and biochemical changes on the rat sciatic/posterior tibial nerve, trigeminal nerve and trigeminal ganglion –**

Official use only

1 REFERENCE

1.1	**References**	**A6.9/02:** [redacted] (1983) Neurotoxicity of WL85871 comparison with WL43467: The effect of 20 oral doses of WL85871 or WL43467 over 4 weeks on the rat sciatic/ posterior tibial nerve, trigeminal nerve and trigeminal ganglion. [redacted], Report no. SBGR.83.185, June 06, 1983 (unpublished). (BASF RDI No.: AL-451-002)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF AG
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
2.2	**GLP**	No
2.3	**Deviations**	Not applicable

3 MATERIALS AND METHODS

3.1	**Test material (a)**	Alphacypermethrin, FASTAC (WL85871)
3.1.1	Lot/Batch number	OCD/7
3.1.2	Specification	Not specified
3.1.3	Purity	94.9%
3.1.4	Description	White powder
3.1.5	Stability	Alphacypermethrin was determined to be stable for 15 months and thus judged to be stable for the duration of the study.
3.2	**Test animals**	
3.2.1	Species	Rat
3.2.2	Strain	Wistar (SPF)
3.2.3	Source	Shell Toxicology Laboratory, Tunstall
3.2.4	Sex	Male and female

Section A6.9 **Neurotoxicity study**
Annex Point IIA 6.9 **– Neurochemical and biochemical changes on the rat sciatic/posterior tibial nerve, trigeminal nerve and trigeminal ganglion –**

3.2.5	Age/weight at study initiation	Age: 11–14 weeks Body weight: 250–410 g (males) 170–270 g (females)
3.2.6	Number of animals per group	Phase I: 5 male and 5 female animals plus additionally 3 male and 3 female "extra" animals for each neurochemical examination time point. Phase II: 10 males and 10 females per group for neurochemical measurements; 10 males and 10 females per group (low and high dose) for neuropathological examinations.
3.2.7	Control animals	Phase I: 5 males and 5 females for each neurochemical examination time point. Phase II: 10 males and 10 females for neurochemical measurements; 10 males and 10 females for neuropathological examinations.
3.3	**Administration/ Exposure**	
3.3.1	Duration of treatment	Phase I: one month Phase II: one month
3.3.2	Frequency of exposure	5 days per week for 4 weeks (except for animals in phase I which were sacrificed after 2 and 3 weeks of treatment).
3.3.3	Post-exposure period	Phase I: until week 12 after commencement of dosing. Phase II: until week 5 after commencement of dosing
3.3.4	Type	Oral (gavage)
3.3.5	Dose levels	Phase I: 37.5 mg/kg Alphacypermethrin in DMSO (week 1 and 2), 25 mg/kg Alphacypermethrin in arachis oil (week 3 and 4) Phase II: 10, 20 and 40 mg/kg Alphacypermethrin in DMSO
3.3.6	Vehicle	DMSO (used for the first two weeks of phase I and phase II); arachis oil (used in second half of phase I).
3.3.7	Concentration in vehicle	10.0%
3.3.8	Stability in vehicle	The test compound was stable in arachis oil for 5 days, and in DMSO for 24 hours.
3.3.9	Total volume applied	Phase I: 0.375 and 0.25 ml/kg Phase II: 0.1, 0.2 and 0.4 ml/kg
3.3.10	Controls	DMSO or arachis oil (2 ml/kg phase I); DMSO (1.5 ml/kg phase II)
3.4	**Examinations**	
3.4.1	Body weight	Phase I: five days per week during the 4 week treatment period; 3 times per week for the treatment free period. Phase II: five days per week during the 4 week treatment period.

Section A6.9 **Neurotoxicity study**
Annex Point IIA 6.9 **– Neurochemical and biochemical changes on the rat sciatic/posterior tibial nerve, trigeminal nerve and trigeminal ganglion –**

3.4.2	Clinical signs	Phase I: every weekday for the first 6 weeks of the study. Thereafter, all surviving animals were observed routinely on a daily basis unless any animal showed any signs of intoxication. Afterwards, formal records were made once per week at the time of body weights measurements. Phase II: every weekday during the dosing period.
3.4.3	Biochemistry	Phase I: 5 males and 5 females were sacrificed 2, 3, 4, 5, 6, 8, and 12 weeks after commencement of treatment for biochemical examination in selected nerve tissue. Phase II: If there were sufficient survivors, 10 males and 10 females per group were sacrificed for biochemical examination after 4 weeks of treatment. Parameters: β-glucuronidase and β-galactosidase activity (expressed as µg Methylumbelliferone liberated) in trigeminal ganglia, trigeminal nerve, proximal and distal section of sciatic posterior tibial nerve.
3.4.4	Pathology	Neuropathology: Phase II: 5 rats per sex bearing the lowest number from the control low and high dose groups were used for neuropathological examination after whole body perfusion at study termination.
3.4.5	Histopathology	Histopathological examination: sciatic and posterior tibial nerve, trigeminal nerve and trigeminal ganglion were prepared and stored for later examination if required.
3.4.6	Statistics	Student's *t*-test or one-way analysis of variance were used for all statistical comparisons.
3.5	**Further remarks**	A range finding test (12.5, 25, 37.5 and 50 mg/kg bw/day Alphacypermethrin) was carried out for dose level selection. In this study, Cypermetrin was examined in parallel, but the data are not shown, because the results did not differ between the two pyrethroids except that higher dose levels were necessary to show the effects.

4 RESULTS

4.1	**Mortality**	Phase I: the dosing regime resulted in the deaths of 23 of the 112 Alphacypermethrin-treated animals (21%). There were more male deaths (17/56) than female deaths (6/56). Phase II: no treatment related deaths occurred. However, one female rat receiving 10 mg/kg and one male rat receiving 20 mg/kg bw/day Alphacypermethrin died. The reason for the lower mortality level in this phase of the study is related to the fact that DMSO was used as the vehicle solvent.

Section A6.9 **Neurotoxicity study**

Annex Point IIA 6.9 **– Neurochemical and biochemical changes on the rat sciatic/posterior tibial nerve, trigeminal nerve and trigeminal ganglion –**

4.2	**Clinical signs**	Phase I: Over 80% of the animals treated with Alphacypermethrin showed signs of intoxication. The most frequently observed clinical signs were gait abnormalities and whole body muscle incoordination. Males and females were equally affected, and the time of onset was observed in proportion of animals from the second day onwards and were still present until day 60. Onset and duration of clinical signs varied from individual to individual. Other clinical signs included piloerection, lethargy, chromodacryorrhea, salivation, hyper-excitability and unkempt appearance. The control animals showed, with the exception of piloerection, none of these signs to any significant extent. Phase II: Clinical signs of intoxication similar to those reported in phase I of the study were seen in the animals administered 20 and 40 mg/kg bw/day Alphacypermethrin. These signs included piloerection, ataxia, "tip toe" walk / splayed hind legs. Other clinical signs observed sporadically included swollen foot, pale eyes and pale skin, abnormal respiratory noise and diarrhoea. The high dose animals showed the highest incidence of clinical signs. Piloerection was seen in control group and 10 mg/kg bw/d group animals as well.
4.3	**Body weight**	Phase I: Alphacypermethrin treated male animals showed no body weight gain over the 4 week dosing period, but reverted to a normal rate of weight gain within 5 weeks of the start of the experiment. Body weight gain of females treated with Alphacypermethrin was similar to the control. Phase II: Male and female rats administered 40 mg/kg bw/day showed no weight gain during the first 2 weeks of dosing and body weight gain remained lower during the second half of the dosing period. Rats of the low and mid dosed groups showed a reduced weight gain during the treatment period, which returned to normal with cessation of dosing.

Section A6.9 **Neurotoxicity study**

Annex Point IIA 6.9 **– Neurochemical and biochemical changes on the rat sciatic/posterior tibial nerve, trigeminal nerve and trigeminal ganglion –**

4.4	**Biochemistry**	
4.4.1	Enzyme activity	Phase I: the β-glucuronidase and β-galactosidase activities in the distal section of the sciatic/posterior tibial nerve of Alphacypermethrin treated male and female rats were statistically significantly increased relative to control at week 5 and 8 after the commencement of dosing. The β-glucuronidase activity in week 6 was also increased, but this was not statistically significant. By the end of the 12-week study, the increased enzyme activity had returned to the control range. The maximal enzyme changes occurred at week 5. No significant enzyme changes were found in the trigeminal ganglia, trigeminal nerve and proximal section of sciatic/posterior tibial nerve at any time during this phase of the study. Results are presented in Table A6.9- 6. Phase II: Several statistically significant changes of the β-glucuronidase and/or β-galactosidase activity in distal and/or proximal sciatic/posterior tibial nerves (SPTN) were observed in animals of the 40 mg/kg bw/d groups. In animals of the 20 mg/kg bw/d group, a small but significant increase in β-galactosidase was found in the distal and proximal section of sciatic/posterior tibial nerves. The magnitude of this effect was comparable with those observed at week 5 in phase I. Results are presented in Table A6.9- 7 and Table A6.9- 8. Statistically significant changes of the β-glucuronidase and/or β-galactosidase activity were also found in trigeminal ganglia and trigeminal nerves of animals treated with 20 and 40 mg/kg bw/d Alphacypermethrin. The largest and most consistent changes were seen in the trigeminal ganglia, but these changes were not dose-related, with the largest increase at 20 mg/kg bw/d. In general, the changes in the trigeminal nerve mirrored the changes in the trigeminal ganglia. There were no differences between animals of the control and the 10 mg/kg bw/d group. Results are presented in Table A6.9- 9 and Table A6.9- 10.
4.5	**Pathology**	
4.5.1	Neuropathology	No evaluation, see 3.4.4.
4.6	**Other**	None

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The experiment was performed in two phases. In the first phase, the time course of neurochemical changes over 12 weeks was determined by measurement of the β-glucuronidase and β-galactosidase activity in the sciatic/posterior tibial nerve (SPTN), trigeminal ganglion and trigeminal nerve of male and female Wistar rats after treatment for 4 weeks (5 days per week) with Alphacypermethrin or Cypermethrin. In phase II of the study, the dose levels of Alphacypermethrin and Cypermethrin that produced no biochemical evidence of peripheral nerve degeneration after administration of the test substances for 4 weeks (5 days per week) were established.

The results for Cypermethrin are not shown in the current study summary.

The study was not a guideline study.

Section A6.9 **Neurotoxicity study**

Annex Point IIA 6.9 **– Neurochemical and biochemical changes on the rat sciatic/posterior tibial nerve, trigeminal nerve and trigeminal ganglion –**

5.2	**Results and discussion**	The results showed that the effects on body weight gain and the incidence of signs of intoxication (i.e. ataxia, gait abnormalities, piloerection) were dose-related. There is no evidence from this study to suggest that Alphacypermethrin induced nerve lesions produce functional or clinical abnormalities. The principal signs are almost exclusively of pharmacological origin and may suggest clinical manifestation of peripheral neuropathy. However, as ataxia and abnormal gait appeared sporadically, did not develop gradually and occurred in some cases within hours after dosing, the time scale argues for a pharmacological effect rather than the consequence of structural damage. The biochemical results of phase I examination showed that the time of maximum change in enzyme activity in the sciatic/posterior tibial nerve (SPTN) was determined to be 5 weeks. The changes were transitory and returned to normal levels within 12 weeks after start of treatment. The relatively small changes were found more consistently in the distal section of the nerves and were judged to be more likely of the Wallerian type, i.e. axonal rather than of the segmental demyelinating type. In the trigeminal ganglia or trigeminal nerve, no significant enzyme changes were found. In phase II, the largest changes in β-glucuronidase and β-galactosidase activities consistent with sparse axonal degeneration were found in the distal section of the sciatic/posterior tibial nerve of groups administered 40 mg/kg bw/day Alphacypermethrin. At 20 mg/kg bw/day, a small but significant increase in β-galactosidase activity was found in the proximal and distal sections of SPTN. Significant, although more sporadic enzyme changes were also found in the trigeminal ganglia and, to a lesser extent, in the trigeminal nerve of the groups administered 20 and 40 mg/kg bw/day. The enzyme changes in the trigeminal nerve and ganglia were smaller than the changes in SPTN. These changes were variable and showed little relationship to the administered dose and would therefore, not be expected to be related to any pathological changes in these tissues. The β-glucuronidase and β-galactosidase activities in animals treated with 10 mg/kg bw/day Alphacypermethrin were not different from the controls. It was concluded that biochemical changes consistent with sparse axonal degeneration were found in the sciatic/posterior tibial nerve (SPTN) of rats which had received 20 or 40 mg/kg bw/day Alphacypermethrin for 4 weeks, supporting a NOAEL of 10 mg/kg bw/day.
5.3	**Conclusion**	
5.3.1	LO(A)EL	20 mg/kg bw/day
5.3.2	NO(A)EL	10 mg/kg bw/day
5.3.3	Reliability	2
5.3.4	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 accepted
Results and discussion	Applicant's version accepted
Conclusion	Applicant's version accepted
Reliability	3 (no guideline, no GLP)
Acceptability	acceptable
Remarks	none
	COMMENTS FROM APPLICANT
Date	4 May 2009
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	Please kindly consider that the reliability indicator should assess the quality of the study, which may be good irrespective of guideline compliance or GLP conformity. These are not key criteria for downgrading the reliability. "3" actually means "*Study with major methodological and/or reporting deficiencies*" according to the TNsG on dossier preparation and would typically result in non-acceptability of a study. Since this is not the case, a reliability indicator of "2" as proposed by the applicant should be maintained.
Acceptability	
Remarks	
	COMMENTS FROM RAPPORTEUR MEMBER STATE
Date	May 2009
Materials and Methods	Applicant's version accepted
Results and discussion	Applicant's version accepted
Conclusion	Applicant's version accepted
Reliability	2 (Applicant's version accepted)
Acceptability	acceptable
Remarks	Remarks of applicant on reliability accepted

Table A6.9- 6: Temporary effect of Alphacypermethrin on the β-glucuronidase and β-galactosidase activity in distal section of sciatic/posterior tibial nerve of rats (male and female).

	β-glucuronidase activity expressed as µg Methylumbelliferone liberated h^{-1} mg (wet weight of nervous tissue)		**β-galactosidase activity expressed as µg Methylumbelliferone liberated h^{-1} mg (wet weight of nervous tissue)**	
Week	**Control[1]**	**Alphacypermethrin[2]**	**Control[1]**	**Alphacypermethrin[2]**
2	0.460 ± 0.034	0.465 ± 0.030 [101%]	1.748 ± 0.117	1.591 ± 0.084 [91%]
3	0.526 ± 0.043	0.507 ± 0.053 [96%]	1.991 ± 0.126	1.738 ± 0.136 [87%]
4	0.477 ± 0.031	0.530 ± 0.053 [111%]	2.024 ± 0.090	2.058 ± 0.156 [102%]
5	0.394 ± 0.025	0.645 ± 0.047 [164%] **	1.288 ± 0.045	1.814 ± 0.130 [141%] *
6	0.438 ± 0.031	0.544 ± 0.049 [124%]	1.833 ± 0.109	2.064 ± 0.115 [113%]
8	0.440 ± 0.032	0.605 ± 0.033 [137%] **	1.219 ± 0.100	1.702 ± 0.108 [140%] **
10	0.399 ± 0.020	0.455 ± 0.026 [114%]	1.153 ± 0.083	1.399 ± 0.091 [121%]
12	0.325 ± 0.022	0.360 ± 0.031 [111%]	1.322 ± 0.075	1.445 ± 0.091 [109%]

1) 11 x 2 ml/kg DMSO and 9 x 2 ml/kg arachis oil

2) cumulative dose of 562.5 mg/kg

3) cumulative dose of 2550 mg/kg

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

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

figures in square brackets = % of mean control values

Table A6.9- 7: Dose-related effect of Alphacypermethrin on the β-glucuronidase and β-galactosidase activity in distal section of sciatic/posterior tibial nerve of rats (male, female and sexes combined).

Dose [mg/kg bw/day]	Distal section of sciatic/posterior tibial nerve		
	M	**F**	**Combined**
β-*glucuronidase*			
Control	0.411 ± 0.017	0.443 ± 0.024	0.427 ± 0.015
10	0.434 ± 0.033 [106%]	0.407 ± 0.025 [92%]	0.420 ± 0.020 [98%]
20	0.434 ± 0.029 [106%]	0.444 ± 0.037 [100.2%]	0.439 ± 0.023 [103%]
40	0.548 ± 0.034*** [133%]	0.566 ± 0.037*** [128%]	0.557 ± 0.025** [130%]
β-*galactosidase*			
Control	0.621 ± 0.024	0.607 ± 0.028	0.614 ± 0.018
10	0.627 ± 0.035 [101%]	0.627 ± 0.046 [103%]	0.651 ± 0.024 [106%]
20	0.708 ± 0.042 [114%]	0.679 ± 0.031 [112%]	0.715 ± 0.037** [116%]
40	0.842 ± 0.088* [136%]	1.331 ± 0.145*** [219%]	1.017 ± 0.086** [166%]

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

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

***) significantly greater than control mean ($p<0.001$)

figures in square brackets = % of mean control values

Table A6.9- 8: Dose-related effect of Alphacypermethrin on the *β*-glucuronidase and *β*-galactosidase activity in proximal section of sciatic/posterior tibial nerve of rats (male, female and sexes combined).

Dose [mg/kg bw/day]	Proximal section of sciatic/posterior tibial nerve		
	M	**F**	**Combined**
β-*glucuronidase*			
Control	0.302 ± 0.017	0.297 ± 0.011	0.299 ± 0.010
10	0.303 ± 0.010 [100.3%]	0.320 ± 0.021 [108%]	0.312 ± 0.012 [104%]
20	0.318 ± 0.015 [105%]	0.295 ± 0.019 [99%]	0.307 ± 0.012 [103%]
40	0.310 ± 0.016 [103%]	0.326 ± 0.024 [110%]	0.318 ± 0.014 [106%]
β-*galactosidase*			
Control	0.489 ± 0.015	0.498 ± 0.020	0.494 ± 0.028
10	0.528 ± 0.013 [108%]	0.516 ± 0.028 [104%]	0.533 ± 0.028 [108%]
20	0.556 ± 0.015** [114%]	0.625 ± 0.028** [126%]	0.571 ± 0.045** [116%]
40	0.517 ± 0.014 [106%]	0.703 ± 0.049** [141%]	0.594 ± 0.039*** [120%]

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

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

***) significantly greater than control mean ($p<0.001$)

figures in square brackets = % of mean control values

Table A6.9- 9: Dose-related effect of Alphacypermethrin on the β-glucuronidase and β-galactosidase activity in the trigeminal ganglia of rats (male, female and sexes combined).

Dose [mg/kg bw/day]	Trigeminal ganglia		
	M	**F**	**Combined**
β-*glucuronidase*			
Control	0.585 ± 0.020	0.601 ± 0.035	0.593 ± 0.020
10	0.639 ± 0.028 [109%]	0.596 ± 0.025 [99%]	0.616 ± 0.019 [104%]
20	0.647 ± 0.032 [111%]	0.651 ± 0.047 [108%]	0.649 ± 0.028 [109%]
40	0.610 ± 0.022 [104%]	0.751 ± 0.035** [125%]	0.681 ± 0.023* [115%]
β-*galactosidase*			
Control	0.821 ± 0.035	0.772 ± 0.086	0.797 ± 0.046
10	0.914 ± 0.039 [111%]	0.938 ± 0.048 [122%]	0.926 ± 0.031* [116%]
20	1.031 ± 0.048** [126%]	1.163 ± 0.063** [151%]	1.097 ± 0.041*** [138%]
40	0.932 ± 0.033* [114%]	1.060 ± 0.054* [137%]	0.996 ± 0.033** [125%]

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

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

***) significantly greater than control mean ($p<0.001$)

figures in square brackets = % of mean control values

Table A6.9- 10: Dose-related effect of Alphacypermethrin on the *β*-glucuronidase and *β*-galactosidase activity in the trigeminal nerve of rats (male, female and sexes combined).

Dose [mg/kg bw/day]	Trigeminal nerve		
	M	**F**	**Combined**
β-*glucuronidase*			
Control	0.380 ± 0.10	0.422 ± 0.023	0.401 ± 0.013
10	0.416 ± 0.018 [109%]	0.460 ± 0.020 [109%]	0.439 ± 0.014 [109%]
20	0.439 ± 0.025 [116%]	0.451 ± 0.034 [107%]	0.445 ± 0.021 [111%]
40	0.404 ± 0.015 [106%]	0.509 ± 0.035 [121%]	0.457 ± 0.020* [114%]
β-*galactosidase*			
Control	0.634 ± 0.023	0.545 ± 0.030	0.589 ± 0.020
10	0.626 ± 0.023 [99%]	0.607 ± 0.026 [111%]	0.616 ± 0.017 [105%]
20	0.705 ± 0.025* [111%]	0.683 ± 0.050* [125%]	0.694 ± 0.028** [118%]
40	0.663 ± 0.020 [105%]	0.623 ± 0.038 [114%]	0.643 ± 0.021 [109%]

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

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

***) significantly greater than control mean ($p<0.001$)

figures in square brackets = % of mean control values

Section A6.10 Mechanistic study

Annex Point IIIA 6.7

	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 conduct of mechanistic studies with Alphacypermethrin to clarify effects reported in toxicity studies is not considered to be required for the following reasons: The critical effect is neurotoxicity, which has been appropriately addressed in the available toxicity studies. Furthermore, two neurotoxicity studies were performed to appropriately address the mechanisms of toxicity.	
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	April, 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 A6.11 **Annex Point IIIA (–)**	**Study on other routes of administration (parental routes)**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible** [ ] **Scientifically unjustified** [X]	
Limited exposure [ ]	**Other justification** [ ]	
Detailed justification:	According to chapter 3 of the TNsG on additional data requirements, studies on other routes of administration are required only in exceptional cases, for example when (i) studies on parental routes may supplement the information received from toxicokinetic studies and give valuable information e.g. in cases when the gastrointestinal absorption of the chemical in question is poor, or when (ii) acute toxicity studies on intraperitoneal, intravenous subcutaneous and intramuscular routes have been conducted, these should also be submitted. Since the available data from conventional routes of administration and the extensive toxicokinetic data base are considered to provide information adequate and sufficient for risk assessment, the conduct of studies on other routes of administration is not considered to be 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	April, 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 A6.12.1 Medical surveillance data on manufacturing plant personnel

Annex Point IIA 6.9.1

Official use only

1 REFERENCE

1.1	**Reference**	**A6.12.1/01:** Western NJ (1984) Report on the assessment of FASTAC exposures during formulation of technical concentrate (TC) and technical material (TM) at Durban, South Africa, September 1983. Shell International Petroleum Maatschappij, The Hague, Netherlands, Report no. HSE.84.003, April 1984 (unpublished), BASF RDI no.: AL-445-002.
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

(NOT APPLICABLE)

3 MATERIALS AND METHODS

3.1	**Substance**	(i) FASTAC TC (technical concentrate), oil damped, containing a minimum of 75% (m/m) of Alphacypermethrin (ii) FASTAC TM (technical material),dry, containing a minimum of 90% (m/m) of Alphacypermethrin
3.2	**Persons exposed**	
3.2.1	Sex	Not stated
3.2.2	Age/ weight	23 to 53 years old; weight not stated
3.2.3	Known diseases	Operator 1, 2 and 3 had no medical history of note. Operator 4: Both lungs on auscultation showed mild expiratory wheeze.
3.2.4	Number of persons	Group I: Operator 1, 2 and 5 (blending supervisor), industrial hygienist, medical officer and safety advisor. Group II: Operator 3 and 4, industrial hygienist
3.2.5	Other information	Operators 1 to 4 were of Zulu origin, the safety advisor and the industrial hygienist were European.
3.3	**Exposure**	Inhalation or dermal
3.3.1	Reason of exposure	Occupational

Section A6.12.1 **Medical surveillance data on manufacturing plant personnel**
Annex Point IIA 6.9.1

3.3.2	Frequency of exposure	Multiple
3.3.3	Overall time period of exposure	Group I: three days (day 1 and 2 handling with technical concentrate (TC); day 3 handling with technical material (TM)) Group II: one day (day 4 handling with technical material (TM))
3.3.4	Duration of single exposure	*Group I* Day 1: 36–54 minutes Day 2: 29–35 minutes Day 3: 36–37 minutes *Group II* Day 4: 126–129 minutes
3.3.5	Exposure concentration/ dose	Day 1: Batch 1 (TC) blended with 632.4 kg of Alphacypermethrin Day 2: Batch 2 (TC) blended with 637.3 kg of Alphacypermethrin Day 3: Batch 3 (TM) blended with 771.4 kg of Alphacypermethrin
3.3.6	Other information	Operator 1 and 2: Full face respirators and PVC aprons (only day 1) Operator 5: Ori-nasal half mask respirator and PVC aprons (only day1) Medical officer and industrial hygienist: No respiratory protective equipment Operator 3: No respiratory protective equipment, but PVC aprons Operator 4: Full face respirator and PVC aprons
3.4	**Examinations**	Assessment of exposures to Alphacypermethrin during the formulation of technical concentrate and technical material.
3.4.1	Monitoring	Atmospheric Alphacypermethrin dust concentration Alphacypermethrin deposition on surfaces Urinary Alphacypermethrin metabolite concentration Affects on respiratory function Affects associated with skin sensations
3.5	**Treatment**	Not applicable, since no severe signs of intoxication were observed during the study.
3.6	**Remarks**	None

4 RESULTS

4.1	**Clinical signs**	On day 2 very localised burning sensations on the face were experienced by two European subjects: the safety advisor (average exposure of 10.7 µg/m^3) and the industrial hygienist (average exposure of 0.6 mg/m^3)

Section A6.12.1 Medical surveillance data on manufacturing plant personnel

Annex Point IIA 6.9.1

4.2	**Results of examinations**	
4.2.1	Air measurements	Group mean personal exposures were analysed using log-probability plots. For day 1 and 2 during tipping the technical concentrate (TC) the total average personal exposures were 2.8 µg/m^3 and 4.9 µg/m^3 and the total average personal exposure for day 3 during formulating of technical material (TM) was 54.1 µg/m^3. The exposures of operators and those physically involved in emptying Alphacypermethrin were greater than for persons in the vicinity who were observing operations. Comparison of the dust level measurements on the cabinet top on day 1 and 2 (0.2 µg/m3 and 3.5 µg/m3, respectively) showed that the local exhaust extraction ventilation reduce the dust concentration by a factor of up to 17. Results are presented in Table A6.12- 1.
4.2.2	Urinary metabolite concentration	The urinary Alphacypermethrin metabolite (cis WL44776) was not detected at theses exposure levels (limit of detection: 0.01 mg/l; equivalent to 0.02 mg/l Alphacypermethrin).
4.2.3	Respiratory functions	Inhalation of Alphacypermethrin concentration of up to 0.155 mg/m^3 did not give rise to alteration in lung function.
4.2.4	Skin sensations	Only on day 2 pin-point burning skin sensations occurred on exposed areas of neck and face of the industrial hygienist (average exposure: 10.7 µg/m^3) and the safety adviser (average exposure: 0.6 µg/m^3). Onset occurred about 2 hours after exposure and slowly diminished over the following 10 hours. This was believed to be the result of direct settling of Alphacypermethrin particles on the skin. Both subjects are white-skinned. The African operators carrying out the dumping who were also exposed to Alphacypermethrin (5.0, 7.5, 11.4 µg/m^3) did not show any sensations attributable to exposure.
4.3	**Effectivity of medical treatment**	Not applicable
4.4	**Outcome**	As the facial sensations are believed to result from small particles settling on exposed skin of the face it is suggested that a transparent face visor is worn to prevent this direct contact. Handling of oil damped Alphacypermethrin technical concentrate (TC) reduced dust concentrations by a factor of up to 30. It is therefore recommended that Alphacypermethrin is supplied for formulation as an oil damped technical concentrate whenever possible, reducing the potential for dust exposure. Dust emissions were reduced by a factor of up to 17 by the use of a tipping cabinet and associated local exhaust ventilation operating so as to create an inward flow of air.
4.5	**Other**	No other significant observations were recorded.

Section A6.12.1
Annex Point IIA 6.9.1

Medical surveillance data on manufacturing plant personnel

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	An assessment of exposures to Alphacypermethrin during the formulation of technical concentrate and technical material on 5 operators, an industrial hygienist and a safety advisor during 4 days. Exposures were assessed by air measurements, urinary metabolite concentration, respiratory functions and skin sensations. The assessment was carried out at Durban, South Africa.
5.2	**Results and discussion**	Two subjects, the medical officer and the industrial hygienist, both caucasian showed facial paresthesiae while the operators with the relatively higher exposures were all Africans and did not show any skin sensation. It was concluded, that the facial paresthesiae in these two cases were due to particles of the active pesticide settling on the skin. No other affects could be observed.
5.3	**Conclusion**	It is recommended that Alphacypermethrin TC was the preferred material for formulation as the handling generated significantly less dust compared with Alphacypermethrin TM. In addition, the application of local exhaust ventilation to control dust emissions is recommended.

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. We could add :"Local exhaust extraction ventilation may reduce dust concentration by a factor up to 17. Oil damping may also reduce dust concentration (and by a factor up to 30)".
Reliability	2
Acceptability	acceptable
Remarks	none
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.12- 1: Group average personal exposures to Alphacypermethrin TC/TM

Day	Operators					Controller		Total group	Sample position
	1	2	3	4	5	Medical officer	Industrial hygienist	Log-probability	Cabinet top
	Concentration of Alphacypermethrin [µg/m³]								
1 (TC)	10.0	4.2	n.a.	n.a.	5.2	0.7	1.2	**2.8**	0.2
2 (TC; without local exhaust ventilation)	7.5	11.4	n.a.	n.a.	5.0	10.7	0.6	**4.9**	3.5
3 (TM)	155.0	51.6	n.a.	n.a.	81.2	n.a.	13.4	**54.1**	7.7
4 (TM)	n.a.	n.a.	0.2	0.7	n.a.	n.a.	0.2	**n.d.**	n.a.

TC technical concentration
TM technical material
n.a. not applicable
n.d. not determined

Section A6.12.2
Annex Point IIA 6.9.2

Direct observation, e.g. clinical cases, poisoning incidents if available
– Human dose-excretion study –

Official use only

1 REFERENCE

1.1	**Reference**	**A6.12.2/01:** Eadsforth CV, Bragt PC, van Sittert NJ (1988) Human dose-excretion studies with pyrethroids insecticides cypermethrin and alphacypermethrin: relevance for biological monitoring. Xenobiotica 18, 603-614 (published), BASF RDI no.: AL-905-051. **Remark:** This reference constitutes the published results from reference A6.2/04.

2 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)

3 MATERIALS AND METHODS

3.1	**Substance**	Alphacypermethrin
3.1.1	Lot/Batch number	Not stated
3.1.2	Specification	Not stated
3.1.2.1	*Description*	Not stated
3.1.2.2	*Purity*	99.8%
3.1.2.3	*Stability*	No information available
3.2	**Type of study**	Human dose-excretion study
3.3	**Method of data collection**	Collection of urine samples
3.4	**Test persons**	
3.4.1	Selection criteria	Academically trained health professional and research workers without any medical objections. None of the volunteers suffered from skin or metabolic disease, nor had a history of hepatic or renal disease. Furthermore the life style of the volunteers was normal with respect to nutrition, smoking and drinking habits and no drugs were used.
3.4.2	Number of persons	6 volunteers
3.4.3	Sex	Male
3.4.4	Age	Not stated
3.4.5	Diseases	Healthy
3.5	**Control subjects**	None

Section A6.12.2
Annex Point IIA 6.9.2

Direct observation, e.g. clinical cases, poisoning incidents if available

– Human dose-excretion study –

3.6	**Exposure**	
3.6.1	Exposure Route	Oral by capsule
3.6.2	Frequency of exposure	Single and repeated
3.6.3	Concentration of test substance	1.25, 2.50 and 3.75 mg Alphacypermethrin per mL corn oil. One capsule contained 200 µl of the solution.
3.6.4	Vehicle	Gelatine capsules
3.6.5	Exposure period	(a) Single oral dose (b) Repeated oral dose: 5 successive days
3.6.6	Post-exposure period	(a) Single oral dose: 3 days (b) Repeated oral dose: 5 days
3.6.7	Samples (sampling time)	Urine (collection of a pre-exposure sample and subsequently over 24 hour periods for up to 4 days (a) and up to 10 days (b))
3.7	**Examinations**	Urinary volume, creatinine excretion and excretion of urinary *cis*-cyclopropane carboxylic acid. Pre- and post-exposure urine was analysed for the *cis*-cyclopropane carboxylic acid by sulphuric acid/methanol methylation, extraction, clean-up and gas-liquid chromatography with electron-capture detection. This method detects both the free acid and its glucuronic acid conjugate. Selected samples were confirmed by mass-spectrometric detection.
3.8	**Further remarks**	In this publication, Cypermetrin was examined in parallel via oral and dermal exposure. The data on Cypermetrin are not shown since the results after the oral application did not differ between the two pyrethroids. The results after the dermal administration did not allow any conclusion as to the concentration of Cypermethrin and its metabolites in the skin or other organs, or the possibility of other routes of metabolism or excretion.

Section A6.12.2
Annex Point IIA 6.9.2

Direct observation, e.g. clinical cases, poisoning incidents if available
– Human dose-excretion study –

4 RESULTS

4.1 Elimination

After single oral administration of Alphacypermethrin, excretion of *cis*-cyclopropane carboxylic acid was rapid. A mean of 43% of the single dose was excreted in urine as *cis*-cyclopropane carboxylic acid (free and conjugated) in the first 24 hour period after dosing, falling to 1–5% on the next day. The relationship between the oral dose levels and the excretion of free and conjugated cyclopropane carboxylic acid over the first 24 hours was clearly dose-related.

After repeated oral dosing of Alphacypermethrin, urinary excretion of free and conjugated *cis*-cyclopropane carboxylic acid was rapid and consistent over the 5 days period with a mean excretion of 49% of the metabolite each day. Within the following five days values were close to the detection limit. About 1–7% (mean 3%) of the dose were excreted on the day after cessation of dosing. Excretion of free and conjugated cyclopropane carboxylic acid was clearly dose-related.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Dose-excretion studies were carried out with 6 volunteers who received a single oral dose of Alphacypermethrin at 0.25, 0.50 and 0.75 mg followed later by repeated oral doses of Alphacypermethrin over five consecutive days. The aim of the studies was to establish a quantitative relationship between a well-controlled oral dose of Alphacypermethrin and the excretion of a metabolite in urine and to determine whether there is accumulation of Alphacypermethrin in the body following repeated oral exposure.

5.2 Results and discussion

Approximately 43% of the dose was excreted in urine as the *cis*-cyclopropane carboxylic acid in the first 24 hour period after single oral dosing. An average of 49% of Alphacypermethrin was excreted in the urine as *cis*-cyclopropane carboxylic acid in each of the subsequent 24 hour periods after repeated oral administration indicating that intake and excretion were in equilibrium after the first dose.

On the days following single and the final repeated administration, the excreted amounts of the metabolites fell rapidly to 1–5% or 1–7% of the dose, respectively. Therefore, no evidence of delayed excretion or accumulation following repeated dosing was observed.

5.3 Conclusion

5.3.1 Reliability: 2

5.3.2 Validity: The study is well documented, meets generally accepted scientific principles and is therefore considered as acceptable.

5.3.3 Deficiencies: No

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	April, 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 A6.12.3 **Health records, both from industry and any other available sources**
Annex Point IIA 6.9.3

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible** [ ] **Scientifically unjustified** [ ]	
Limited exposure [ ]	**Other justification** [X]	
Detailed justification:	All reasonable effort was undertaken to locate such health records in the applicant's archives. However, such data are not available.	
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	April, 2009
Evaluation of applicant's justification	Applicant's version accepted
Conclusion	Applicant's version accepted
Remarks	none
	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A6.12.4 **Annex Point IIA 6.9.4**	**Epidemiological studies on the general population, if available**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible** [ ] **Scientifically unjustified** [ ]	
Limited exposure [ ]	**Other justification** [X]	
Detailed justification:	A comprehensive literature search on published data was performed recently. However, studies relevant to the current data requirement could not be identified. Moreover, the applicant is not aware of other potential sources for such 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	April, 2009	
Evaluation of applicant's justification	Applicant's version accepted	
Conclusion	Applicant's version accepted	
Remarks	none	
	COMMENTS FROM ...	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A6.12.5
Annex Point IIA 6.9.5

Diagnosis of poisoning including specific signs of poisoning and clinical tests

Official use only

1 REFERENCE

1.1 Reference

A6.12.5/01:
Tordoir (1990): Occupational health aspects of handling pesticides in Shell Agrochemical formulation plants – A guide for occupational health advisers. Shell International Petroleum Maatschapij, The Hague, Netherlands, Report no. HSE 90.009, May 1990 (published), BASF RDI no.: AL-445-005.

2 GUIDELINES AND QUALITY ASSURANCE

(NOT APPLICABLE)

3 MATERIALS AND METHODS

(NOT APPLICABLE)

4 RESULTS

(NOT APPLICABLE)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Test substance

Alphacypermethrin is a cyano pyrethroid which evokes a stronger response in human beings than non-cyano pyrethroids. Pyrethroids are highly active insecticides and are often used in conjunction with other chemicals which enhance their effectiveness.

5.2 Type of exposure

Ingestion, inhalation or dermal absorption of Alphacypermethrin can lead to local effects or systemic effects when handling the concentrated products found within the formulation plants, laboratories and testing stations.

5.3 Mode of action

From toxicological studies it is known that pyrethroids act on the nervous system and produce excitatory effects predominantly on the sensory nerve endings. In extreme cases this may lead to blockage of the transmission of nerve impulses.

Alphacypermethrin is very lipophilic and dose-excretion studies in humans demonstrated rapid metabolisation and elimination from the body.

Skin absorption in humans is very limited.

Section A6.12.5 **Diagnosis of poisoning including specific signs of poisoning and clinical tests**
Annex Point IIA 6.9.5

5.4	**Pyrethroid Poisoning**	
5.4.1	Signs and symptoms	There are reports of acute intoxications in field workers leading to signs and symptoms such as dizziness, headache, nausea, anorexia, fatigue, gastro-intestinal complaints and fever. In severe cases exposure resulted in impaired consciousness, muscular fasciculation, convulsions, coma and pulmonary oedema.
5.4.2	Local effects	The primary route of occupational exposure in formulation plants is by skin contact. Skin sensations occur at the exposed parts of the body which quite often is the face. These skin sensations appear to be transient and usually wear off after a few hours, although longer periods have been reported. There is no evidence of any change in the sensory function of the affected skin. Inhalation my lead to temporary irritation of the upper respiratory tract. Response varied from rhinorrhoea to chest tightness and dyspnoea.
5.4.3	Systemic effects	Ingestion of pyrethroids poses the major hazard and may lead to effects on the central nervous system leading to a variety of symptoms ranging from dizziness and nausea to fasciculation and convulsions. Aspiration of the liquid formulation into the lungs resulted in chemical pneumonitis. It is not clear if, and to what extent, systemic effects can be expected in case of skin contact or inhalation. From animal and human dose excretion studies it is known that penetration through the skin and absorption via the inhalation route is low. There is no evidence that the central nervous system is affected in workers exposed to low doses of pyrethroids for a long period of time. Most cases of acute systemic poisoning reported appear to have been the result of accidental or intentional ingestion. The absolute number of such cases is however small.
5.5	**Treatment**	Symptomatic treatment is recommended in cases of acute intoxication. There is no specific antidote. Convulsions should be treated with anti-convulsants. It is not clear if atropine medication is beneficial. It may even be dangerous if a clear diagnosis has not been established. Mephenesis is known to reduce symptoms and fatalities in animal studies. As there is virtually no experience with this compound with regard to human intoxications, the use of this drug is not recommended. In case of ingestion, gastric lavage should be applied as soon as possible.

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 accepted
Results and discussion	Applicant's version accepted
Conclusion	Applicant's version accepted
Reliability	1
Acceptability	acceptable
Remarks	none
	COMMENTS FROM ...
Date	
Materials and methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.12.6 Sensitisation / allergenicity observations, if available

Annex Point IIA 6.9.6

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible** [ ] **Scientifically unjustified** [ ]	
Limited exposure [ ]	**Other justification** [X]	
Detailed justification:	A comprehensive literature search on published data was performed recently. However, studies relevant to the current data requirement could not be identified. Moreover, the applicant is not aware of other potential sources for such data. Thus, it is concluded that observations on sensitisation or allergenicity are not available. Furthermore, Alphacypermethrin has clearly been shown not to elicit any skin sensitising effects in a Magnusson & Kligman type study (section A6.1.5).	
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	April, 2009	
Evaluation of applicant's justification	Applicant's version accepted	
Conclusion	Applicant's version accepted	
Remarks	none	
	COMMENTS FROM ...	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A6.12.7
Annex Point IIA 6.9.7

Specific treatment in case of an accident or poisoning: first aid measures, antidotes and medical treatment

Official use only

1 REFERENCE

1.1 Reference

A6.12.7/01:
Flannigan SA, Tucker SB (1985) Variation in cutaneous sensation between synthetic pyrethroids insecticides. Contact Dermatitis 13: 140–147 (published), BASF RDI no.: AL-905-072.

2 GUIDELINES AND QUALITY ASSURANCE

(NOT APPLICABLE)

3 MATERIALS AND METHODS

(NOT APPLICABLE)

4 RESULTS

(NOT APPLICABLE)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Test substances

Four widely utilised synthetic pyrethroids:

Cypermethrin ([S,R]-alpha-cyano-3-phenoxybenzyl-2,2-dimethyl[IR,IS,cis,trans]-3-(2,2-dichlorovinyl) cyclo-propane-carboxylate)

Fenvalerate (Cyano (3-phenoxyphenyl) methyl 4-chloro-alpha-(1-methylethyl)benzeneacetate)

Flucythrinate ((RS)-alpha-cyano-3-phenoxybenzyl (S)-2-(4-difluromethoxyphenyl)-3-methylbutyrate

Permethrin ((3-phenoxyphenyl)methyl (±) cis-trans 3-(2-2-dichloroethenyl)-2-2-dimethyl-cyclopropanecarboxylate

Composition: Active ingredient (32–36%), organic solvents and surfactants (inert ingredients).

5.2 Treatment

Examination applied to the induced paresthesia from the topical treatment of the formulated pyrethroids. Applications of 0.05 cc of field strength formulated pyrethroids (each 0.13 mg/cm^2) or the inert ingredients were delivered to a 4 cm^2 area on the lower margin of one earlobe of each participant on 5 separate occasions. The opposite earlobe received 0.05 cc of distilled water. Participant evaluation after each application continued for 48 hours and involved grading and describing any unusual cutaneous sensation.

Section A6.12.7
Annex Point IIA 6.9.7

Specific treatment in case of an accident or poisoning: first aid measures, antidotes and medical treatment

5.2.1	Administration of therapeutic drug	An evaluation was made of the therapeutic capability of topical Vitamin E Acetate (d1-alpha tocopheryl acetate) to ameliorate the paresthesia that accompanies cutaneous exposure to the formulated pyrethroids. Therefore 0.05 cc of Vitamin E Acetate were applied to the earlobe after treatment with pyrethroids.
5.3	**Results**	All four pyrethroids induced paresthesia: Permethrin resulted in the least amount of paresthesia (0.15), Cypermethrin and Fenvalerate were approximately equal (0.59; 0.57), Flucythrinate produced the greatest response (1.20). No cutaneous sensation was reported by any of the participants to the inert ingredients when applied separately. A statistically significant difference in sensation was apparent between the inert ingredients and all the synthetic pyrethroids except Permethrin. In each case the paresthesia developed with a latency period of approximately 30 min, peaked by 8 hours and deteriorated as early as 24 hours. Topical application of Vitamin E Acetate inhibited the induced sensation of all of the four formulated pyrethroids (therapeutic index: 89–100%). Therefore it was found to be a highly efficacious therapeutic agent for synthetic pyrethroid exposure.

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 accepted
Results and discussion	Applicant's version accepted
Conclusion	Applicant's version accepted
Reliability	1
Acceptability	acceptable
Remarks	None
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.12.7 **Specific treatment in case of an accident or poisoning: first aid measures, antidotes and medical treatment**
Annex Point IIA6.9.7

Official use only

1 REFERENCE

1.1 Reference

A6.12.7/02:
Hiromori T, Nakanishi T, Kawaguchi S, Sako H, Suzuki T, Miyamoto J (1986) Therapeutic effects of methocarbamol on acute intoxication by pyrethroids in rats. J. Pestic. Sci.11: 9–14, 1986 (published), (BASF RDI no.: AL-905-074).

2 GUIDELINES AND QUALITY ASSURANCE

(NOT APPLICABLE)

3 MATERIALS AND METHODS

(NOT APPLICABLE)

4 RESULTS

(NOT APPLICABLE)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Test substances

Four widely utilised synthetic pyrethroids:

Cypermethrin [(*RS*)-α-cyano-3-phenoxybenzyl (1*RS*)-*cis*, *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate]

Fenvalerate [(*RS*)- α-cyano-3-phenoxybenzyl (*RS*)-2-(4-chlorophenyl)-3-methylbutyrate]

Fenpropathrin [(RS)- α-cyano-3-phenoxybenzyl 2,2,3,3-tetramethylcyclopropanecarboxylate]

Permethrin [3-phenoxybenzyl (1RS)-cis, trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate]

(Purity:92–96%)

5.2 Treatment

Pyrethroids dissolved in corn oil were administered orally to male Sprague-Dawley rats (5 mL/kg bw) at a dose level which could cause mortality higher than 50% (Fenvalerate: 850 mg/kg; Cypermethrin: 850 mg/kg; Fenpropathrin: 100 mg/kg; Permethrin: 850 mg/kg). Then the toxic symptoms were observed at appropriate intervals for 5–7 days and every 30 minutes when the symptoms developed severely.

Section A6.12.7 **Specific treatment in case of an accident or poisoning: first aid measures, antidotes and medical treatment**
Annex Point IIA6.9.7

5.2.1	Administration of therapeutic drug	An atropine sulphate solution (25 mg dissolved in 1 mL of physiological saline) was subcutaneously injected at a dose of 25 mg/kg bw when salivation was observed after administration of Fenvalerate and Cypermethrin. The same dose was administered afterwards whenever salivation reappeared. Methocarbamol was given intraperitoneally when tremor or hyperexcitability to sound was observed, initially at a dose of 400 mg/kg bw followed by repeated doses of 200 mg/kg on every onset of the symptoms.
5.3	**Results**	The incidence of toxic symptoms (tremor, choreoathetosis, fibrillation, hyperexcitability or salivation) produced by the pyrethroids were statistically significantly reduced by a single or repeated doses of Methocarbamol, except for fibrillation caused by Fenopropathrin and Permethrin. Moreover, the mortality caused by massive doses of pyrethroids decreased statistically significantly to 0% and for Permethrin from 70% to 10% after administration of Methocarbamol. Administration of Atropine sulphate also prevented profuse salivation caused by Fenvalerate and Cypermethrin. Details are presented in Table A6.12.7- 1. Animals administered 400 mg/kg Methocarbamol exhibited ataxia, limb paralysis, a loss of righting reflex and irregular respiration at the early stage of the intoxication. They recovered within 6 hours after the administration. Those administered 600 mg/kg Methocarbamol died. The study presented some basic information on therapeutic measures for sublethal or lethal intoxication in men suffered from a massive exposure to pyrethroids.

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 accepted
Results and discussion	Applicant's version accepted
Conclusion	Applicant's version accepted
Reliability	1
Acceptability	acceptable
Remarks	None
COMMENTS FROM ...	
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.12.7- 1: Therapeutic effects of Methocarbamol and Atropine sulphate on acute intoxication by pyrethroids in male rats (10 per group).

Pyrethroid (dose, mg/kg)	Drugs	Average frequency of treatments	Toxic symptoms (%)					Mortality (%)
			Sa	F	T	H	C	
Fenvalerate (850)								
	–	–	100	60	100	90	90	80
	MET	2.6	100	0**	0**	0**	0**	0**
	AT	1.0	0**	70	100	100	100	80
	AT/MET	1.1/4.7	0**	0**	0**	0**	0**	0**
Cypermethrin (850)								
	–	–	100	0	90	60	100	80
	MET	6.5	100	0	10**	10*	0**	0**
	AT	1.7	0**	0	50	60	90	90
	AT/MET	1.2/6.0	0**	0	0**	0*	0**	20*
Fenpropathrin (100)								
	–	–	0	0	100	50	10	60
	MET	4.0	0	10	10**	0*	0	0**
Permethrin (850)								
	–	–	0	90	100	0	0	70
	MET	5.2	0	100	50*	0	0	10**

Sa	salivation
F	fibrillation
T	tremor
H	hyperexcitability
C	clonic seizure and/or choreoathetotic
MET	Methocarbamol
AT	Atropine sulfate
*	$p<0.05$ statistically significant difference
**	$p<0.01$ statistically significant difference

Section A6.12.8 Prognosis following poisoning

Annex Point IIA 6.9.8

Official use only

1 REFERENCE

1.1	**Reference**	**Cross-references to:** **A5.4/01:** van Heemstra-Lequin EAH, van Esch GT (1992) Alphacypermethrin. Environmental Health Criteria 142, WHO ICPS, Geneva, Switzerland (published), BASF RDI No.: AL-901-012. **A6.2/04:** [redacted] (1984) Human oral dose-excretion study with Fastac. [redacted], Report no. HSE 85.010, November 1984 (unpublished), BASF RDI no.: AL-445-003. **A6.12.1/01:** Western NJ (1984) Report on the assessment of FASTAC exposures during formulation of technical concentrate (TC) and technical material (TM) at Durban, South Africa, September 1983. Shell International Petroleum Maatschappij, The Hague, Netherlands, Report no. HSE.84.003, April 1984 (unpublished), BASF RDI no.: AL-445-002. **A6.12.2/01:** Eadsforth CV, Bragt PC, van Sittert NJ (1988) Human dose-excretion studies with pyrethroids insecticides cypermethrin and alphacypermethrin: relevance for biological monitoring. Xenobiotica 18, 603-614 (published), BASF RDI no.: AL-905-051. **A6.12.5/01:** Tordoir (1990): Occupational health aspects of handling pesticides in Shell Agrochemical formulation plants – A guide for occupational health advisers. Shell International Petroleum Maatschapij, The Hague, Netherlands, Report no. HSE 90.009, May 1990 (published), BASF RDI no.: AL-445-005. **A6.12.7/01:** Flannigan SA, Tucker SB (1985) Variation in cutaneous sensation between synthetic pyrethroids insecticides. J. Agric. Food Chem. 26, March 1985 (published), BASF RDI no.: AL-905-072.
1.2	**Data protection**	Yes (A6.2/04, A6.12.1/01)
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	None
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I of Directive 98/8/EC.

2 GUIDELINES AND QUALITY ASSURANCE

(NOT APPLICABLE)

Section A6.12.8 **Prognosis following poisoning**
Annex Point IIA 6.9.8

3 MATERIALS AND METHODS

(NOT APPLICABLE)

4 RESULTS

(NOT APPLICABLE)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Review of relevant information given in the cross-references.

5.2 Results and discussion

From a review of the information available from the references cited in section 1.1 above, the following conclusions may be drawn:

Ingestion, inhalation or dermal absorption of Alphacypermethrin can lead to local or systemic effects when handling the concentrated products found within the formulation plants, laboratories and testing stations.

It is generally known that pyrethroids act on the nervous system and produce excitatory effects predominantly on the sensory nerve endings. In extreme cases this may lead to blockage of the transmission of nerve impulses (e.g. A5.4/01, A6/12.5/01).

Dose-excretion studies in humans (oral single and repeated administration) demonstrated that Alphacypermethrin is readily metabolised and rapidly (49% within 24 h) eliminated from the body (A6.2/04, A6.12.2/01). There was no evidence whatsoever of delayed excretion and/or accumulation.

Ingestion

Acute oral toxicity is high when administered in a non-polar vehicle (LD_{50} = 57 mg/kg bw, rat), whereas the substance is in principle non-toxic when administered in a polar vehicle (LD_{50} > 2000 mg/kg bw, rat).

Ingestion of pyrethroids may lead to effects on the central nervous system, leading to a variety of symptoms ranging from dizziness and nausea to fasciculation and convulsions.

There is, however, no evidence that the central nervous system is affected in workers exposed to low doses of pyrethroids for a long period of time.

Most cases of acute systemic poisoning reported appear to have been the result of accidental or intentional ingestion. The absolute number of such cases is however small.

Inhalation

Acute inhalation toxicity tests resulted in a lowest LC_{50} of 1.33 mg/l air.

Section A6.12.8 Prognosis following poisoning

Annex Point IIA 6.9.8

		Dermal Alphacypermethrin is of low acute dermal toxicity in the rat (LD_{50} > 2000 mg/kg bw, see section A6.1.2), and skin absorption in humans is very limited. Typical adverse effects in humans due to dermal exposure to Alphacypermethrin are described as paresthesia (A6.12.1/01, A6.12.7/01). Treatment Symptomatic treatment is recommended in cases of acute intoxication. There is no specific antidote. Convulsions should be treated with anti-convulsants. It is not clear if atropine medication is beneficial. It may even be dangerous if a clear diagnosis has not been established. Mephenesis is known to reduce symptoms and fatalities in animal studies. As there is virtually no experience with this compound with regard to human intoxications, the use of this drug is not recommended. In case of ingestion, gastric lavage should be applied as soon as possible.
5.3	**Conclusion**	Acute systemic exposure to Alphacypermethrin may elicit symptoms on the CNS, whereas acute dermal exposure may lead to paraesthesia or burning skin sensations. However, in both cases the symptoms are of transient nature, usually declining within 10–24 hours, which is consistent with the rapid metabolism and elimination of Alphacypermethrin. Acute cases of poisoning may therefore be assumed not to result in enduring adverse effects. This is confirmed by experience with plant personnel. Furthermore, apart from the generally known symptoms of paraesthesia or burning skin sensation, no cases of acute poisoning were identified in a recent literature search.

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	acceptable
Remarks	none
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.13 Toxic effects on livestock and pets

Annex Point IIIA 6.2

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible** [ ] **Scientifically unjustified** [X]	
Limited exposure [X]	**Other justification** [ ]	
Detailed justification:	From the intended use as a domestic insecticide, exposure of livestock and pets to Alphacypermethrin is considered to be unlikely. In view of the limited exposure, livestock and pets are not expected to be at risk. However, in view of the well-known mechanisms of toxicity (see section A6.9), information on toxicokinetics and the well established metabolism in mammals as well as the large number of studies on toxicology in mammals, extrapolation from the available information to livestock and pets is not considered to be restricted in any way. It is also noted that this study type is neither a common core data requirement, nor a product-type specific additional data requirement according to the TNsG. Thus, in conclusion, the conduct of toxicity study on livestock and pets is not considered to be 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	April, 2009
Evaluation of applicant's justification	Applicant's version accepted
Conclusion	Applicant's version accepted
Remarks	None
	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A6.14 Other tests related to the exposure of humans

Annex Point IIIA 11.2

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible** [ ] **Scientifically unjustified** [ ]	
Limited exposure [ ]	**Other justification** [X]	
Detailed justification:	Other tests related to the exposure of humans are not considered to be required, since the TNG on additional data requirements expressly requires such information for example only under the following prerequisites: (i) in the case of toxicity of degradation products, by-products and reaction products related to human exposure (ii) in the case of toxic effects of substances generated from an active substance, other than mammalian metabolites, in the normal use of a biocidal product (iii) for product types that may involve relevant human exposure due to reaction products with water when the substance is used for human hygiene purposes or reaction products with water or other materials released in water or air when the substance is used for the treatment of bathing waters, for example. However, none of these considerations apply to the product type 18 (insecticides) and especially not to Alphacypermethrin, which is why any such tests are not considered to be 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	April, 2009	
Evaluation of applicant's justification	Applicant's version accepted	
Conclusion	Applicant's version accepted	
Remarks	None	
	COMMENTS FROM ...	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A6.15.1–6 Food and feeding stuffs

Annex Point IIIA 6.4, 11.1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9

JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Technically not feasible** [ ] **Scientifically unjustified** [ ] **Limited exposure** [X] **Other justification** [X]	
Detailed justification: (i) The submission of data on residues in food and feeding stuffs is not considered to be required for lack of exposure, since the intended use as a domestic insecticide (predominantly crack and crevice treatment) and the related application practices of the formulated products are in no way associated with any potential for contamination of food and feeding stuffs. The biocidal product is not intended to be applied to surfaces that may come in contact with food. In chapter 3, points A6.15.1–6.15.5 of the TNsG on data requirements the submission of such data is only requested in the case that "the active substance is to be used in preparations for use where food for human consumption is prepared, consumed or stored, or where feeding stuff for livestock is prepared, consumed or stored." However, the application technique and the nature of the treated areas by insecticides for domestic/public hygiene clearly exclude the possibility of food contact. (ii) Data to be submitted under point 6.15.1–6.15.6 are not part of the mandatory "common core data" set for active substances, but instead represent either product-type specific "additional data requirements" as further specified in chapter 2.5 of the TNsG on data requirements, or further additional data requirements as set forth in chapter 3 of the TNsG on data requirements. To this, it is initially noted that for insecticides such as Alphacypermethrin, such additional data are not required according to chapter 2.5 or chapter 3. (iii) For the reasons above, a summary under point A6.15.6 similarly does not need to be submitted.	
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	April, 2009	
Evaluation of applicant's justification	Applicant's version accepted	
Conclusion	Applicant's version accepted	
Remarks	None	
	COMMENTS FROM ...	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A6.16 **Annex Point IIIA 6.3.5, 11.2**	**Any other tests related to the exposure of the active substance to humans, in its proposed biocidal products, that are considered necessary may be required**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible** [ ] **Scientifically unjustified** [ ]	
Limited exposure [ ]	**Other justification** [X]	
Detailed justification:	In chapter 3 of the TNsG on additional data requirements it is stated that it may be required under point A6.16 to submit other tests related to the exposure of the active substance to humans in its proposed biocidal products that are considered necessary. Further, it is said that an expert judgement for suitable tests and reasoned case is needed to decide whether such additional studies are required. The document then refers to chapter 1.2, point 4, where it also says: The data requirements have been specified in as much detail as possible. However, in certain cases **expert judgement** by the applicant and by the competent authority may be necessary in order to assess, for instance, whether an additional study is needed or on which organism or under which conditions a test should be performed. The applicant hereby states that a dossier is submitted which is largely congruent with the one agreed on EU level in the context of Directive 91/414 for the inclusion of Alphacypermethrin in Annex I. In fact, there have been some additions to the underlying data base in order to address biocide-specific issues. Since there is agreement among EU Member States that the data base is adequate to positively conclude on inclusion in Annex I of Directive 91/414, and since all essential data requirements have also been met concerning Directive 98/8, the applicants does not at this time see any need for further data submissions.	
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	April, 2009	
Evaluation of applicant's justification	Applicant's version accepted	
Conclusion	Applicant's version accepted	
Remarks	None	
	COMMENTS FROM ...	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A6.17
Annex Point IIIA 6.6

If the active substance is to be used in products for action against plants then tests to assess toxic effects of metabolites from treated plants, if any, where different from those identified in animals shall be required

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible** [ ] **Scientifically unjustified** [ ]	
Limited exposure [ ]	**Other justification** [ ]	
Detailed justification:	In chapter 3 of the TNsG on additional data requirements it is stated under point A6.17 that the submission tests to assess toxic effects to metabolites from treated plants may be required, if the active substance is to be used in products for action against plants. However, since Alphacypermethrin is exclusively used as an insecticide, the conduct of such tests is obviously not relevant.	
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	April, 2009	
Evaluation of applicant's justification	Applicant's version accepted	
Conclusion	Applicant's version accepted	
Remarks	None	
	COMMENTS FROM ...	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A7.1.1.1.1
Annex Point IIA 7.6.2.1

Hydrolysis as a function of pH and identification of breakdown products

Official use only

1 REFERENCE

1.1	**Reference**	**A7.1.1.1.1/01:** van Dijk A (1993) Hydrolysis determination of ^{14}C-alphacypermethrin at different pH values. RCC, Itingen, Switzerland, Report no. 307383, December 10, 1993, BASF RDI No.: AL-322-002 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	None
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 111 (1981) 79/831 EEC Method C.7
2.2	**GLP**	Yes
2.3	**Deviations**	Yes Different concentrations and concentrations above the saturation concentration of the test substance in water were used.

3 MATERIALS AND METHODS

3.1	**Test material**	As given in Section A
3.1.1	Lot/Batch number	S 0819
3.1.2	Specification	As given in Section A.
3.1.3	Purity	> 99.8% radiochemical purity Spec. radioactivity 25.6 mCi/g (0.947 MBq/mg)
3.1.4	Further relevant properties	Water solubility at 20°C: pH 4 4.59 µg/L pH 7 5.80 µg/L pH 9 7.87 µg/L Distilled water 2.06 µg/L Vapour pressure: 3.4×10^{-7} Pa at 25 °C.

Section A7.1.1.1.1 Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA 7.6.2.1

3.2	**Reference substance**	No	
3.2.1	Initial concentration of reference substance	Not applicable	
3.3	**Test solution**	**Buffer solutions**: pH 4: 0.1 M phosphate buffer pH 7: 0.1 M Tris-buffer pH 9: 0.1 M borate buffer Data on the test solutions are given in Table A7.1.1.1.1-1, replications and experimental conditions (see table A7.1.1.1.1-2)	
3.4	**Testing procedure**		
3.4.1	Test system	The incubations were performed in the dark to avoid photolytical effects and nitrogen was bubbled through for 5 min before the labelled test substance was added. For equipment and sterilisation method please refer to table A7.1.1.1.1-3.	
3.4.2	Temperature	Experiments were run at different temperatures: pH 4: 50°C pH 7: 50, 60 and 75°C pH 9: 25 and 50°C	X
3.4.3	pH	**pH 4** initial: 4.1 final: 4.2 **pH 7** initial: 7.00 final: 7.02 **pH 9** initial: 9.0 final: 9.0	
3.4.4	Duration of the test	The test durations were different depending on temperature and pH, please refer to sampling (3.4.6).	
3.4.5	Number of replicates	One replicate for each sampling interval.	
3.4.6	Sampling	The sampling intervals were different depending on temperature and pH. **pH 4, 50°C** 3, 10 d **pH 7, 50°C** 3, 5, 7 and 10d **pH 7, 60°C** 0, 2, 4, 7, 9 and 11d **pH 7, 75°C** 1, 2, 3 and 4d **pH 9, 25°C** 0, 1, 2, 3, 4, 7.2 and 11d **pH 9, 50°C** 0, 1, 2, 3, 4, 6, 8, 10 and 24h	X

Section A7.1.1.1.1 **Hydrolysis as a function of pH and identification of breakdown products**
Annex Point IIA 7.6.2.1

3.4.7	Analytical methods	**Normal and reversed phase TLC** Precoated silica gel plates (5 * 20 cm * 0.25 mm) Normal Phase 60 F 254 (Merck, Germany) Reversed phase RP-18 F 254 (Merck, Germany) **Solvent systems:** SS5: Chloroform /n-hexane (80+20) NP SS8: Acetonitril/ H_2O (90+10) RP Without chamber saturation **Detection:** Labelled samples: 100–200 µL (1,500–2,500 dpm) were applied and detected by using a Berthold Automatic TLC-Linear Analyser (LB 2842). Non-labelled reference compounds: 10µg/10µL in acetone were visualised by UV-light at 254 nm. **Sample preparation pH 4 and 7** - 15 mL aliquots cooled to room temperature, - acidification to pH 1 (4M HCl) - extraction 2-3 times with ethylacetate (1+2, v/v) - The combined ethylacetate phases were dried over sodium sulphate and concentrated under reduced pressure at 35°C to 1-2 mL. - rel. recoveries: 70.6 – 91.3% **Sample preparation pH 9** - 300 mL aliquots cooled to room temperature, - acidification to pH 1 (4M HCl) - extraction 2 times with hexane - The combined hexane phases were concentrated under reduced pressure at 35°C to about 1 mL. - rel. recoveries: 80.9–91.5% (2 exceptions 67.5%) Duplicate analysis of same buffer solution (extract).	
3.5	**Preliminary test**	Yes, at 50°C and pH4, pH7 and pH9 using 0.2 mg/L. pH 4: 1–7 days pH 7: 3–7 days pH 9: 0–4 hours The results were inconsistent with respect to percentage parent as well as percentage total recovery. Additionally, artefactual effects occurred on normal phase TLC. Therefore, the concentration of the test item was lowered at least 10 fold in the main test.	

4 RESULTS

4.1	**Concentration and hydrolysis values**	In the aqueous solution at pH 4, exclusively the parent compound was found until 10 days of incubation at 50°C (total recovery 83–88%). The hydrolysis results for the other pH levels and observation times for parent compound, and transformation products are presented in tabular form (see table A7.1.1.1.1-4-8).	X

Section A7.1.1.1.1 **Hydrolysis as a function of pH and identification of breakdown products**
Annex Point IIA 7.6.2.1

4.2	**Hydrolysis rate constante (k_h)**	**pH 7:** 50°C: k = 0.0257 d^{-1}, r = –0.9985 60°C: k = 0.132 d^{-1}, r = –0.9829 75°C: k = 0.3388 d^{-1}, r = –0.9953 ln k = 19.749 – 7250.8 + 1/T **pH 9:** 25°C: k = 0.0083 h^{-1}, r = –0.9966 50°C: k = 0. 2337 h^{-1}, r = –0.9920 ln k = 38.266 – 12837.6 + 1/T
4.3	**Dissipation time**	See table A7.1.1.1.1-9.
4.4	**Concentration-time data**	The nominal concentration of test substance was 0.02 mg/l and 0.001mg/L, respectively. Concentration of the parent compound and the transformation products expressed as percentage of initial concentrations are given in table A7.1.1.1.1-4 to A7.1.1.1.1-8.
4.5	**Specification of the transformation product**	See table A7.1.1.1.1-10.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The hydrolytic stability of Alphacypermethrin was tested in accordance with the OECD guideline 111 at pH 4, 7, and 9 at different temperatures in phosphate, Tris- and borate buffer solutions. Solutions of 4.6 mg /L and 0.792 mg /L Alphacypermethrin in acetone were diluted with buffer solutions, so that solutions of 0.02 mg /L and 0.001 mg /L Alphacypermethrin are resulted containing 0.4 and 0.1% acetone. The buffer solutions were incubated in tightly closed vessels in a water bath of given temperature. At every sample interval one glas vessel was extracted and analysed. The study shows no significant deviations from the OECD guideline.
5.2	**Results and discussion**	The half-lives and hydrolysis rate constants of Alphacypermethrin for pH 7 and 9 at the different temperatures are given in table A7.1.1.1.1-9. At pH 4, the hydrolytic degradation was so slow at 50°C, that a half-live above 1 year (25°C) can safely be assumed. The half-lives at 20°C and 25°C were determined graphically via extrapolation by plotting ln k against 1/T (T = absolute temperature). Results are summarised below.
5.2.1	k_H	pH7 (20°C): 0.00685; (25°C): 0.01036 pH9 (20°C): 0.00399; (25°C): 0.0083
5.2.2	DT_{50}	pH4 (25°C): > 1 year pH7 (20°C): 101 d; (25°C): 67d pH9 (20°C): 7.3 d; (25°C): 3.5d

Section A7.1.1.1.1
Annex Point IIA 7.6.2.1

Hydrolysis as a function of pH and identification of breakdown products

5.2.3	*r^2*	–
5.3	**Conclusion**	Validity criteria can be considered as fulfilled. Hydrolysis of Alphacypermethrin takes place at neutral and quickly at alkaline pH. The major degradation product was identified as 3-phenoxybenzaldehyde.
5.3.1	Reliability	2
5.3.2	Deficiencies	No, the following deviations occurred but these are not considered as deficiencies: The concentrations for the preliminary test and main test pH 4 and pH 7 (0.2mg/L and 0.02mg/L) were above the saturation concentration of the test substance in water. Different concentrations were used for pH 4 / pH 7 and pH 9.

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	February 2009
Materials and Methods	The Applicant's version is considered to be acceptable with the following comments: Section 3.4.2: According to the Guideline OECD 111, the test has to be carried out at 3 different temperatures and pH. However, in the applicant's version only 2 temperatures were tested at pH 9. Section 3.4.6: According to the Guideline OECD 111, the duration of the test is 30 days or 90% of hydrolysis. However, in this study the duration of the test does not exceed 11 days and no clear explanation is provided by the applicant.
Results and discussion	The Applicant's version is considered to be acceptable with the following amendment: Section 4.1 Tables A7.1.1.1.1-4-5-6-7-8 and A7.1.1.1.1-A-B-C-D-E => see tables at pages 10-11-12 of this document).
Conclusion	The Applicant's version is considered to be acceptable
Reliability	2
Acceptability	Acceptable
Remarks	As reported by the applicant in section 5.3.2 of this document, minor deviations have occurred in this study: the test was performed at only 2 temperatures instead of 3 at pH 9 and the duration of the definitive study is reduce at 11 days instead of 30 days. However, BE CA thinks that these deviations are not going to interfere with the validity of the results.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	