Section A6.1.1 Acute Oral Toxicity - Rat

Annex Point IIA 6.1 **Rat, cypermethrin, oral LD50**

		Oral
3.3.2	Type	Gavage
3.3.3	Concentration	300 mg/kg bodyweight (G1F first and second treatment step) and 2000 mg/kg bodyweight (G2F first treatment step)
3.3.4	Vehicle	Refined groundnut oil
3.3.5	Concentration in vehicle	60 mg/ml (GF1 300 mg/kg group) and 400 mg/ml (GF2 2000 mg/kg group)
3.3.6	Total volume applied	5 ml/kg bw
3.3.7	Controls	Not applicable
3.4	**Examinations**	Toxic signs and mortality observed 5 times during day 1 (at 30 mins and 4 times at hourly intervals) and once daily during days 2-15. Bodyweights recorded pre-administration (day 1) and 8, 15 days post treatment/death. Gross necropsy at death and all survivors at the end of the observation period.
3.5	**Method of determination of LD_{50}**	According to Annex 2c of guideline 423
3.6	**Further remarks**	-
		4 RESULTS AND DISCUSSION
4.1	**Clinical signs**	No toxic signs/pre-terminal deaths in the G1F (300 mg/kg) group. In the G2F (2000 mg/kg) group, toxic signs observed were slight/severe salivation, tremors, lethargy, ataxia and perineum wet with urine.
4.2	**Pathology**	In the G2F group, one rat died on day 2 and no abnormality was detected at necropsy. The other 2 rats died on day 3, lung congestion was detected in both rats at necropsy. No abnormalities detected in remaining rats at necropsy.
4.3	**Other**	In the G1F group, all rats gained bodyweight during the observation period. In the G2F group, all dead rats lost weight compared to their initial bodyweight.
4.4	**LD_{50}**	LD50 was found to be **500 mg/kg bw** as per LD50 cut-off value of Annex 2c of the guideline. Category 4 as per Globally harmonized classification system of Annex 2c of the guideline.

Section A6.1.1 Acute Oral Toxicity - Rat

Annex Point IIA 6.1

Rat, cypermethrin, oral LD50

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Acute oral administration in rat (acute toxic class method) with 15 day post-treatment observation based on OECD guideline 423. A dose of 300 mg/kg bw (G1F group) was administered to 3 female rats on day1. Three further animals were treated at the same dose 3 days later. Based on the results, the next upper dose of 2000 mg/kg bw was administered to 3 animals to determine the LD50 cut-off value.
5.2	**Results and discussion**	An LD50 of 500 mg/kg was determined based on the acute toxic class method.
5.3	**Conclusion**	Acute oral LD50 = 500 mg/kg bw
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April, 2011.
Materials and Methods	The applicant's version is acceptable.
Results and discussion	The applicant's version is adopted with the following amendment: 4.1 Clinical signs: G1F (300 mg/kg) group: tremors observed in one rat on day 1. No toxic signs were observed in the remaining rats.
Conclusion	The applicant's version is adopted. **Acute oral LD50 = 500 mg/kg bw**
Reliability	1
Acceptability	Acceptable
Remarks	

Section A6.1.1 Acute Oral Toxicity - Rat

Annex Point IIA 6.1

Rat, cypermethrin, oral LD50

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_1_1. Table for Acute Oral Toxicity

Dose [mg/kg]	*Number of dead / number of investigated*	*Time of death (range)*	*Observations*
G1F 1st treatment 300 mg/kg	0/3	-	~~No toxic sign~~Tremors observed in 1 animal on day 1.
G1F 2nd treatment 300 mg/kg	0/3	-	No toxic sign
G2F 2000 mg/kg	3/3	Day 2 or 3	Slight/severe salivation, tremors, lethargy, ataxia and perineum wet with urine
LD_{50} value	500 mg/kg bw		

Section A6.1.2 Acute Dermal Toxicity - Rat

Annex Point IIA 6.1.2

Rat, cypermethrin, dermal LD50 (limit test)

			Official use only
		1 REFERENCE	
1.1	**Reference**	Kobel, W (1984); Acute Dermal LD50 in the Rat of CGA 55186 Tech. (cypermethrin); Ciba-Geigy Ltd, report No.:840045 (CYP/T 82f), 9 April 1984 (unpublished). Dates of work: 14 February 1984 – 2 May 1984	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Chimac-Agriphar s.a.	
1.2.2			
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I authorisation	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes Existing study partially conforming to method B.3. of Directive 92/69/EEC (OECD guideline 402).	
2.2	**GLP**	No GLP was not compulsory at the time the study was performed	
2.3	**Deviations**	Yes Description of necropsy findings are missing. Substance should normally be applied under semi-occluded dressing.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	CGA 55186 tech (cypermethrin cis:trans/40:60)	
3.1.1	Lot/Batch number	307046	
3.1.2	Specification	Deviating from specification given in section 2 as follows	
3.1.2.1	Description	Liquid	
3.1.2.2	Purity	92.6%	
3.1.2.3	Stability	Guaranteed by original sponsor (Ciba-Geigy Ltd)	X
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Tif:RAIF (SPF), F3-crosses of RII 1/Tif x RII 2/Tif	
3.2.3	Source	Ciba-Geigy Ltd, Tierfarm, 4334 Sisseln, Switzerland	
3.2.4	Sex	Males and females	
3.2.5	Age/weight at study initiation	7-8 weeks, 179-224 g	
3.2.6	Number of animals per group	5 males, 5 females	
3.2.7	Control animals	No	

Section A6.1.2 Acute Dermal Toxicity - Rat

Annex Point IIA 6.1.2 **Rat, cypermethrin, dermal LD50 (limit test)**

3.3	**Administration/ Exposure**	Oral	X
3.3.1	Post exposure period	14 days or until all symptoms disappeared	
		Dermal	
3.3.2	Area covered	10% of body surface	
3.3.3	Occlusion	Dermal gauze lined occlusive dressing	
3.3.4	Vehicle	None	
3.3.5	Concentration in vehicle	2000 mg/kg bw	
3.3.6	Total volume applied	Linear to dose	
3.3.7	Removal of test substance	Water (lukewarm)	
3.3.8	Controls	None	
3.4	**Examinations**	Mortality recorded twice daily (once daily on weekends), clinical observations daily, body weight recorded on days 1, 7, 14 and at death. Gross necropsy at death and all survivors at the end of the observation period.	
3.5	**Method of determination of LD_{50}**	Logit method (J. Berkson, J.Am.Stat. Ass. 39. 357-65, 1944): LD50 including the 95% confidence limit.	
3.6	**Further remarks**		
		4 RESULTS AND DISCUSSION	
4.1	**Clinical signs**	No mortalities in either males or females. Clinical observations included dyspnoea, ruffled fur, curved and ventral body position. A transient diarrhoea was observed. All animals recovered within 10 days. No reaction at site of application.	
4.2	**Pathology**	No gross lesions found at necropsy.	
4.3	**Other**		
4.4	**LD_{50}**	LD50 in both sexes >2000 mg/kg bw	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Dermal occlusive application in rat of 2000 mg/kg cypermethrin for 24 hours with 14 day post-treatment observation (based on OECD guideline 402).	
5.2	**Results and discussion**	No mortalities were observed. An LD50 of >2000 mg/kg (with 95% confidence limits) was determined for both sexes.	
5.3	**Conclusion**		
5.3.1	Reliability	2	

Section A6.1.2	**Acute Dermal Toxicity - Rat**
Annex Point IIA 6.1.2	**Rat, cypermethrin, dermal LD50 (limit test)**

5.3.2 Deficiencies	Yes Detailed description of necropsy findings are missing and the test substance should be applied under semi-occluded dressing. Considering that no mortalities were observed and all animals recovered within 10 days, this is not thought to influence the acceptability of the study. Study has been previously evaluated and accepted under Directive 91/414/EC.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March 2007.
Materials and Methods	The applicant's version is adopted with following amendments: 3.1.2.3 Stability: … but data not shown. 3.3 Administration/exposure: Dermal, one single dose applied onto the skin of the back.
Results and discussion	The applicant's version is adopted with the following amendment: Table A6.1.2_1: Table for acute dermal toxicity.
Conclusion	LD50 of >2000 mg/kg (with 95% confidence limits) determined for both sexes.
Reliability	2
Acceptability	acceptable (Although the study was performed before GLP and despite the limited enquiries, the protocol was based on OECD guideline No. 402 and EC test method B3.)
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.1.2_1: Table for Acute Dermal Toxicity

Dose [mg/kg]	*Number of dead / number of investigated*	*Time of death (range)*	*Observations*
2000 (limit dose)	0/10	-	Dyspnoea, ruffled fur, ventral and curved body position. Transient diarrhea. All animals recovered by day 10. **No** local reaction on the site of application.
LD_{50} value	> 2000 mg/kg bw		

Section A6.1.3 Acute Inhalation Toxicity - Rat

Annex Point IIA 6.1.3 **Rat, cypermethrin, Inhalation Exposure (LC50)**

			Official use only
		1 REFERENCE	
1.1	**Reference**	Bretz, R (1985); Acute Aerosol Inhalation Toxicity in the Rat of CGA 55186 Tech. (cypermethrin); Ciba-Geigy Ltd, report No.:840047 (CYP/T82g), 2 May 1985 (unpublished) Dates of experimental work: 31 July 1984 – 5 December 1984	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Chimac-Agriphar s.a.	
1.2.2			
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I authorisation	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes Existing study with protocol based on method B.2. of Directive 92/69/EEC (corresponding OECD guideline 403)	
2.2	**GLP**	No GLP was not compulsory at the time the study was performed	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	CGA 55186 tech (cypermethrin cis:trans/40:60)	
3.1.1	Lot/Batch number	307046	
3.1.2	Specification	Deviating from specification given in section 2 as follows	
3.1.2.1	Description	Liquid, viscous	
3.1.2.2	Purity	92.6%	
3.1.2.3	Stability	Stable at room temperature	X
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Tif: RAI f (SPF), F3-crosses of RII 1/Tif x RII 2/Tif	
3.2.3	Source	Ciba-Geigy Ltd	
3.2.4	Sex	Males and females	
3.2.5	Age/weight at study initiation	Young adults, 229±29g (males) and 212±13g (females)	
3.2.6	Number of animals per group	5 males, 5 females	
3.2.7	Control animals	Yes	

Section A6.1.3 Acute Inhalation Toxicity - Rat

Annex Point IIA 6.1.3 **Rat, cypermethrin, Inhalation Exposure (LC50)**

3.3	**Administration/ Exposure**	Inhalation	
3.3.1	Postexposure period	14 days	
		Inhalation	
3.3.2	Concentrations	Nominal concentration : 0, 2217, 3119, 3478, 5170 mg/m^3 Analytical concentration: 0, 970, 1926, 3462, 5328 mg/m^3	
3.3.3	Particle size	MMAD 1.6-2.9 µm, 82-94% of airborn particles had a diameter smaller than 7 µm.	X
3.3.4	Type or preparation of particles	Aerosol generated in a pneumatic nebulizer	
3.3.5	Type of exposure	Nose only	
3.3.6	Vehicle	Ethanol	
3.3.7	Concentration in vehicle	20 % w/w cypermethrin	
3.3.8	Duration of exposure	4 hours	
3.3.9	Controls	Ethanol (nominal concentration 32.4 g/m$^{3)}$	
3.4	**Examinations**	Mortality and clinical symptoms observed during exposure at 1, 2 and 4 hours, as well as 2 hours after exposure and then daily thereafter for 14 days. Dead animals removed twice daily on working days. Body weight recorded on days 7, 14 and at death. Gross necropsy at death and on all survivors at the end of the observation period.	
3.5	**Method of determination of LD_{50}**	Logit method (J. Berkson, J.Am.Stat. Ass. 39. 357-65, 1944)	
3.6	**Further remarks**	-	
		4 RESULTS AND DISCUSSION	
4.1	**Clinical signs**	Dyspnoea, sedation, exophthlamos, ruffled fur, curved and ventral body position, tremor, convulsions were seen in both sexes to a similar extent but with a dose-dependent increase in intensity and duration. In addition, the high dosed males showed extreme irritability and hyperkinetic behaviour. Surviving animals recovered within 9 days.	X
4.2	**Pathology**	About half of the animals in the two higher dose groups showed mottled, hemorrhagic, or edematous lungs, as well as dilatation of the stomach. In 2 males and 1 female exposed to 3462 mg/m^3, dilatations of the heart were found.	
4.3	**Other**	*Body weight*: male rats showed significantly lower weight gain during the first week after exposure and compensated with increased gain in the second week. Females were not significantly affected.	
4.4	**LD_{50}**	LC_{50} male : 3281 mg/m^3 (= 291 mg/kg bw) LC_{50} female : 5038 mg/m^3 (= 448 mg/kg bw) LC_{50} : 3894 mg/m^3 (= 327 mg/kg bw)	X

Section A6.1.3 **Acute Inhalation Toxicity - Rat**

Annex Point IIA 6.1.3 **Rat, cypermethrin, Inhalation Exposure (LC50)**

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Acute (4 hr) inhalation exposure in rat with 14 day post-treatment observation, based on EC method B.2.
5.2	**Results and discussion**	An LC50 of 3894 mg/m^3 (with 95% confidence limits) was calculated for both sexes.
5.3	**Conclusion**	
5.3.1	Reliability	1
5.3.2	Deficiencies	No, study was evaluated and accepted under Directive 91/414/EC.

Section A6.1.3 Acute Inhalation Toxicity - Rat

Annex Point IIA 6.1.3 **Rat, cypermethrin, Inhalation Exposure (LC50)**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March, 2007.
Materials and Methods	The applicant's version is adopted with the following amendments: 3.1.2.3 Stability: stable at room temperature. Guaranteed by sponsor, but data not shown. 3.3.3 Particle size: MMAD 1.6-2.9 µm, GSD 2.3-2.7, 82-94% of airborn particles with diameter < 7 µm (w/w).
Results and discussion	The applicant's version is adopted with the following amendments: 4.1 Clinical signs and Mortality: In the test groups, dyspnea, ruffled fur, curved body position, and convulsions were observed for both sexes to a similar extent but with a dose-dependent increase in intensity and duration. In addition, the high dosed males showed extreme irritability and hyperkinetic behaviour. Surviving animals recovered within 9 days. In the control (ethanol) group, sedation, dyspea, exophthalmos, and ruffled fur were observed at the day of application. All deaths occurred during the exposure period or within 2 hours thereafter. LC_{50} male: 3281 (2181-7102) mg/m³ air LC_{50} female: 5038 (lower: 2640) mg/m³ air LC_{50} both sexes: 3894 (2884-6607) mg/m³ air Table A6.1.3_1: Table for Acute Inhalation Toxicity
Conclusion	An LC50 (4 h) of 3894 mg/m^3 (with 95% confidence limits) was calculated for both sexes. Nevertheless, the LC50 (4h) males = 3281 mg/m³ will be used for risk characterization purposes.
Reliability	2 (not GLP)
Acceptability	acceptable
Remarks	

Section A6.1.3 Acute Inhalation Toxicity - Rat

Annex Point IIA 6.1.3

Rat, cypermethrin, Inhalation Exposure (LC50)

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.1.3_1: Table for Acute Inhalation Toxicity

Conc. [mg/km³]	*Number of dead / number of investigated*	*Time of death (range)*	*Observations*
0	0/10 (♂ 0/5;♀ 0/5)	-	Dyspnea, exophthalmus, ruffled fur, sedation. All animals recovered 1 day post-exposure.
970	0/10 (♂ 0/5;♀ 0/5)	-	Dyspnea, ruffled fur, curved body position. All animals recovered by day 5.
1926	1/10 (♂ 0/5;♀ 1/5)	Within 2 hours after exposure	Dyspnea, ruffled fur, curved body position. All animals recovered by day 7.
3462	3/10 (♂ 2/5;♀ 1/5)	During exposure	Dyspnea, ruffled fur, curved body position, convulsions. All animals recovered by day 9.
5328	8/10 (♂ 5/5;♀ 3/5)	During exposure and within 2 hours after expsore	Dyspnea, ruffled fur, curved body position, convulsions. All animals recovered by day 7.
LD_{50} value	1945 (1449-2676) mg/kg bw		

Section A6.1.4e Acute Eye Irritation -Rabbit

Annex Point IIA 6.1.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	Seifert, G (1984); Acute Eye Irritation / Corrosion Study in the Rabbit of CGA 55186 Tech. (cypermethrin); Ciba-Geigy Ltd, report No.:840043 (CYP/T82i), 15 March 1984 (unpublished)	
		Dates of work: 16 February 1984 – 2 March 1984	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Chimac-Agriphar s.a.	
1.2.2			
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I authorisation	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes	
		Protocol in compliance with method B.5. of Directive 92/69/EEC (corresponding OECD guideline 404, adopted 12 May 1981)	
2.2	**GLP**	No	
		GLP was not compulsory at the time the study was performed	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	CGA 55186 tech (cypermethrin cis:trans/40:60)	
3.1.1	Lot/Batch number	307046	
3.1.2	Specification	Deviating from specification given in section 2 as follows	
3.1.2.1	Description	Liquid, viscous	
3.1.2.2	Purity	92.6% w/w	
3.1.2.3	Stability	Stable	X
3.2	**Test Animals**		
3.2.1	Species	Rabbit	
3.2.2	Strain	New Zealand white	
3.2.3	Source	Kleintierfarm Madoerin AG, Germany	
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	12-14 weeks, 2050-2180 g	
3.2.6	Number of animals per group	3	
3.2.7	Control animals	No (left eye of each animal left untreated as a control)	

Section A6.1.4e Acute Eye Irritation -Rabbit

Annex Point IIA 6.1.4

3.3	**Administration/ Exposure**	
3.3.1	Preparation of test substance	Test substance was used as delivered
3.3.2	Amount of active substance instilled	0.1 ml
3.3.3	Exposure period	Test item administered only once
3.3.4	Postexposure period	11 days
3.4	**Examinations**	
3.4.1	Ophthalmoscopic examination	No
3.4.1.1	Scoring system	Cornea opacity, iris, conjunctiva and chemosis scored according to the grading system described in method B.5. of Directive 92/69/EEC)
3.4.1.2	Examination time points	60min, 24h, 48h, 72h, and again during the following observation period with slit-lamp
3.4.2	Other investigations	None
3.5	**Further remarks**	-
		4 RESULTS AND DISCUSSION
4.1	**Clinical signs**	Slight to severe irritation and swelling
4.2	**Average score**	According to EU methodology:
4.2.1	Cornea	24+48+72 h =0/0/0
4.2.2	Iris	24+48+72 h = 0/0/0
4.2.3	Conjunctiva	
4.2.3.1	Redness	24+48+72 h = 2/1.3/1.6
4.2.3.2	Chemosis	24+48+72 h = 1/0.33/0.66
4.3	**Reversibility**	Yes All effects reversed by the end of the 11 day observation period
4.4	**Other**	All animals showed normal body weight development
4.5	**Overall result**	Irritant but not corrosive
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Acute eye irritation/corrosion in the rabbit of 0.1 ml cypermethrin
5.2	**Results and discussion**	Cypermethrin is irritant but not corrosive to rabbit eye
5.3	**Conclusion**	Cypermethrin is slightly irritant but does not require classification
5.3.1	Reliability	1
5.3.2	Deficiencies	No. Study was evaluated and accepted under Directive 91/414/EC

Section A6.1.4e Acute Eye Irritation -Rabbit

Annex Point IIA 6.1.4

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March, 2007.
Materials and Methods	The applicant's version is acceptable with the following amendments: 3.1.2.3 Stability: data not shown.
Results and discussion	Revision of results: See table A6_1_4_E-1. Results of eye irritation study.
Conclusion	The applicant's version is adopted: Cypermethrin is slightly irritant but does not require classification.
Reliability	1
Acceptability	acceptable
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.1.4e Acute Eye Irritation -Rabbit

Annex Point IIA 6.1.4

Appendix

Table A6_1_4_E-1. Results of eye irritation study

	Cornea	Iris	Conjunctiva	
			redness	chemosis
score	0 to 4	0 to 2	0 to 3	0 to4
Average score (EU methodology) 24h+48h+72h for each animal	**0/0/0**	**0/0/0**	**2/1.33/1.67**	**1/0.33/0.67**
Individual data:				
60 min	0/0/0	0/0/0	3/3/3	2/3/3
24 h	0/0/0	0/0/0	3/2/3	1/2/2
48 h	0/0/0	0/0/0	2/1/1	1/0/0
72 h	0/0/0	0/0/0	1/1/1	1/0/0
7 days	0/0/0	0/0/0	1/1/0	0/0/0
11 days	0/0/0	0/0/0	0/0/0	0/0/0
Area effected				
Maximum average score (including area affected, max 110)				
Reversibility*	c	c	c	c
average time for reversion				
Give method of calculation maximum average score. * *c :* *completely reversible* *n c :* *not completely reversible* *n :* *not reversible*				

Section A6.1.4s Acute Dermal Irritation - Rabbit

Annex Point IIA 6.1.4 (02)

			Official use only
		1 REFERENCE	
1.1	**Reference**	Seifert, G (1984); Acute Dermal Irritation / Corrosion Study in the Rabbit of CGA 55186 Tech. (cypermethrin); Ciba-Geigy Ltd, report No.:840044 (CYP/T82h), 12 March 1984 (unpublished) Dates of experimental work: 16 February 1984 – 27 February 1984	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Chimac-Agriphar s.a.	
1.2.2			
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I authorisation.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, protocol based on OECD guideline no. 404 (adopted 12 May 1981), corresponding method B.4. of Directive 92/69/EEC.	
2.2	**GLP**	No GLP was not compulsory at the time the study was performed.	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	CGA 55186 tech (cypermethrin cis:trans/40:60).	
3.1.1	Lot/Batch number	307046	
3.1.2	Specification	Deviating from specification given in section 2 as follows	
3.1.2.1	Description	Liquid, viscous	
3.1.2.2	Purity	92.6%	
3.1.2.3	Stability	Stable	X
3.2	**Test Animals**		
3.2.1	Species	Rabbit	
3.2.2	Strain	White New Zealand	
3.2.3	Source	Kleintierfarm Madoerin AG, Germany	
3.2.4	Sex	Male	
3.2.5	Age/weight at study initiation	12-14 weeks, 1980-2150 g	
3.2.6	Number of animals per group	3	
3.2.7	Control animals	No	
3.3	**Administration/ Exposure**	Dermal	

Section A6.1.4s Acute Dermal Irritation - Rabbit

Annex Point IIA 6.1.4 (02)

3.3.1	Application		
3.3.1.1	Preparation of test substance	Test substance was used as delivered.	
3.3.1.2	Test site and Preparation of Test Site	Not less than 24 hours before treatment, an area of 6 cm² was shaved on the back of each animal.	
3.3.2	Occlusion	Occlusive	
3.3.3	Vehicle	None	
3.3.4	Concentration in vehicle	Not applicable, test item was administered as delivered	
3.3.5	Total volume applied	0.5 ml	
3.3.6	Removal of test substance	Dressing removed and skin observed. Removal of test substance not mentioned in report.	
3.3.7	Duration of exposure	4 hours	
3.3.8	Postexposure period	7 days	
3.3.9	Controls	None	
3.4	**Examinations**		
3.4.1	Clinical signs	No	
3.4.2	Dermal examination	Yes	
3.4.2.1	scoring system	Scoring system for erythema and eschar formation: No erythema = 0 Very slight erythema = 1 Well defined erythema = 2 Moderate or severe erythema = 3 Severe erythema to slight eschar formation = 4 Corrosion = + Scoring system for oedema formation: No oedema = 0 Very slight oedema = 1 Slight oedema = 2 Moderate oedema (approx. 1 mm) = 3 Severe oedema (> 1 mm, extended beyond applic. site)) = 4	X
3.4.2.2	Examination time points	1h, 24h, 48h, 72h, 7 days	
3.4.3	Other examinations	Bodyweight	
3.5	**Further remarks**	-	

Section A6.1.4s Acute Dermal Irritation - Rabbit

Annex Point IIA 6.1.4 (02)

		4 RESULTS AND DISCUSSION	
4.1	**Average score**		
4.1.1	Erythema	Average score (EU methodology) for all animals at 24, 48, 72 h = 1.3, 1.3, 2	X
4.1.2	Edema	Average score (EU methodology) for all animals at 24, 48, 72 h = 1, 1, 2	X
4.2	**Reversibility**	Yes All animals recovered after the 7 day observation period	
4.3	**Other examinations**	All animals showed normal body weight development. No other reactions observed.	
4.4	**Overall result**	Cypermethrin is slightly irritant and not corrosive	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Acute (4h) irritation/corrosion to rabbit skin using 0.5 ml cypermethrin. Study in compliance with EC method B.4 of Directive 92/69/EEC (corresponding OECD guideline 404)	
5.2	**Results and discussion**	Animals showed very slight to well defined erythema and very slight oedema. All animals recovered within 7 days.	
5.3	**Conclusion**		
5.3.1	Reliability	1	
5.3.2	Deficiencies	No. Study evaluated and accepted under Directive 91/414/EC.	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March, 2007.

Section A6.1.4s Acute Dermal Irritation - Rabbit

Annex Point IIA 6.1.4 (02)

Materials and Methods	The applicant's version is acceptable with the following amendments: *3.1.2.3 Stability*: data no shown *3.4.2.1 Scoring system, mode of calculation and interpretation*: **The index of primary cutaneous irritation** was calculated as follows: The scores obtained for erythema and oedema at 1, 24, 48, and 72 hours after removal of the patch on the 3 rabbits examined are summed up. The sums of total oedema and erythema were divided by 12 (when all the animals survived). The score obtained is defined as the index of primary cutaneous irritation. Interpretation: Index < 0.5 : not irritant 0.6 – 3.0 : slightly irritant 3.1 – 5.0 : irritant 5.1 – 8.0 : severely irritant
Results and discussion	Revision of results: See table A6_1_4S-1. The calculated dermal irritation index was 2.0.
Conclusion	Cypermethrin is slightly irritant and not corrosive when applied to the rabbit skin. Cypermethrin does not require classification.
Reliability	1
Acceptability	acceptable
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.1.4s Acute Dermal Irritation - Rabbit

Annex Point IIA 6.1.4 (02)

Table A6_1_4S-1. Table for skin irritation study

score	time	Erythema	Edema
average score (EU methology)/ animal	***24h, 48h, 72h***	***1.33/1.33/2.0***	***0.33/0.33/0.67***
Individual score (Draize scores)	60 min	0/2/2	0/1/1
	24 h	2/2/2	1/1/1
	48 h	1/1/2	0/0/1
	72 h	1/1/2	0/0/0
other times	7 days	0/0/0	0/0/0
reversibility: *		c	c
average time for reversibility		7 days	72 h
Index of primary cutaneous irritation		2.0	

* c : completely reversible
n c : not completely reversible
n : not reversible

Section A6.1.5 Skin sensitisation -

Annex Point IIA6.1.5 **Mouse Local Lymph Node Assay (LLNA)**

			Official use only
		1 REFERENCE	
1.1	**Reference**	Robertson, A. (2006): Cypermethrin cis:trans/40:60: local lymph node assay in the mouse (individual method); Covance Laboratories Ltd., report no. 1669/032, 3 March 2006 (unpublished). Dates of experimental work: 3 January 2006 – 31 January 2006	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Chimac-Agriphar s.a.	
1.2.2			
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD guideline 429 (April 2002), method B42 of Directive 2004/73/EC and EPA OPPTS 870.2600 (2003)	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	
3.1.1	Lot/Batch number	SL25163S63	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description	Viscous liquid	
3.1.2.2	Purity	93.05%	
3.1.2.3	Stability	Stable	X
3.1.2.4	Preparation of test substance for application	Vehicle for the test article was 80% v/v acetone in olive oil. Formulations were freshly prepared on days 1, 2 and 3 and were mixed by multiple inversion of the containers prior to use.	
3.1.2.5	Pretest performed on irritant effects	Yes. Concentrations of 50%, 25%, 10%, 5%, 2.5% and 1% v/v cypermethrin in acetone/olive oil (4:1 v/v) were tested in a preliminary screening test.	

Section A6.1.5 **Skin sensitisation -**
Annex Point IIA6.1.5 **Mouse Local Lymph Node Assay (LLNA)**

3.2	**Test Animals**	
3.2.1	Species	Mouse
3.2.2	Strain	CBA/CaCrl
3.2.3	Source	Charles River (UK) Ltd
3.2.4	Sex	Female (non-pregnant, nulliparous)
3.2.5	Age/weight at study initiation	Approximately 8 to 10 weeks old, weighing between 17 and 22g on day before dosing.
3.2.6	Number of animals per group	5
3.2.7	Control animals	Yes (vehicle control and positive control)
3.3	**Administration/ Exposure**	
3.3.1	Induction schedule	Epidermal induction was carried out on three occasions (days 1, 2 and 3). On day 6 all animals were injected (intravenous) with 20 µCi ^{3}H-methyl thymidine in PBS. After approximately 5 hours all animals were killed (CO_2) and the auricular lymph nodes removed from each ear for examination.
3.3.2	Way of Induction	Epidermal (no dressing applied). The outer part of both pinnae of each mouse was treated by direct application of the test or control formulation (0.025 ml/pinna) dispensed from an automatic micro pipette
3.3.3	Concentrations used for induction	Three test substance concentrations were used in the main study. Three groups of 5 animals were treated with either 2.5%, 1% or 0.5% test substance in 80% acetone in olive oil. A vehicle control group was also treated.
3.3.4	Positive control substance	Yes. A positive control group was treated wit 25% α—hexylcinnamaldehyde formulated in acetone/olive oil (4:1 v/v).
3.4	**Examinations**	
3.4.1	Pilot study	Yes. A preliminary irritation study was performed in order to select the highest concentration in the main study. Six mice were used, 1 mouse for each dose level tested (50%, 25%, 10%, 5%, 2.5%, 1.0% and 0.5% v/v respectively)
3.4.2	Recovery of Lymph nodes	The lymph nodes collected into each petri dish were cut open and disaggregated by squashing the fragments with a sharp blade. The resultant liquor was transferred into code-identified conical tubes. The petri dishes were rinsed with an additional 5 mL phosphate buffered saline and the second liquor was added to the first liquor. At each transfer, debris such as fragments of capsule were retained in the petri dish wherever possible. After 5 minutes the pooled liquor was filtered (200µm) and centrifuged at 190 G for 10 minutes. Following centrifugation, the supernatant was discarded and the pellet resuspended in 5 mL phosphate buffered saline. This was centrifuged again and the pellet resuspended in 3 mL of 5% w/v aqueous trichloroacetic acid. The suspension was refrigerated for 18 hours at nominally 4°C. On the following day the suspension was re-centrifuged and the pellet resuspended in 1 mL 5% w/v aqueous trichloroacetic acid, which was

Section A6.1.5 **Skin sensitisation -**
Annex Point IIA6.1.5 **Mouse Local Lymph Node Assay (LLNA)**

then subjected to ultrasonic dispersion for 25 minutes to ensure a homogenous suspension. The suspension (1 mL) was transferred to a scintillation vial and scintillation fluid (*ca* 10 mL) was added.

3.4.3 Scintillation counting

All vials, including the background samples, were submitted for liquid scintillation counting for 10 minutes, using a ^{3}H quench curve. Incorporation of $^{3}HTdR$ is measured by ß-scintillation counting as disintegrations per minute (DPM) over a ten-minute period. This value was corrected to account for the background containing 5% w/v aqueous trichloroacetic acid and scintillation fluid. The DPM value was transformed into a mean DPM value for each group. The mean DPM value for each test group was divided by the mean DPM for the control group to provide the Stimulation Index (SI) value for each test group.

3.5 Further remarks

The test result is not valid for those groups producing an SI value of 3.0 or more when the sites of application have shown excessive irritation and for those groups that have shown indications of systemic toxicosis. The test article is regarded as a sensitiser when the maximum value of the SI is 3.0 or above.

The test article is classified as a non-sensitiser when the maximum value of the SI is less than 3.0. (This result is unchanged by observations of irritation at sites of application of the test formulation).

4 RESULTS AND DISCUSSION

4.1 Results of pilot studies

Signs of systemic toxicity were noted in the mice treated with the 50%, 25%, 10% and 5% v/v concentrations. The mouse treated with the 50% v/v concentration and the mouse treated with the 25% v/v concentration appeared to be very agitated and were writhing approximately five hours after dose administration on Day 1. The mouse treated with the 10% v/v concentration and the mouse treated with the 5% v/v concentration appeared to be slightly agitated approximately five hours after the dose administration on Day 2. The degree of agitation increased and was accompanied by twitching, involuntary spasms and sudden movements within approximately 1 hour. These four animals were killed for humane reasons.

Clinical signs in the two mice treated with, respectively, the 2.5% v/v and 1% v/v concentrations were restricted to greasiness to the head, neck and ears. Based on this information the dose levels selected for the main test were 2.5%, 1% and 0.5% v/v in 80% v/v acetone in olive oil.

4.2 Results of test

4.2.1 Clinical signs

There were no clinical signs indicative of a systemic effect of treatment among mice treated with the vehicle or with 0.5, 1.0 or 2.5% v/v formulations of Cypermethrin *cis:trans* 40:60. However, all groups displayed greasiness on the head and neck on all six days of the observation period and all animals treated with the 2.5% v/v concentration showed slight reddening of the head, ears and neck on Days 2, 3 and 4. This sign was also noted in the positive controls from Day 2 until the end of the observation period.

All animals survived treatment with the test article.

Section A6.1.5 **Skin sensitisation -**
Annex Point IIA6.1.5 **Mouse Local Lymph Node Assay (LLNA)**

4.2.2	Stimulation indices	See Table A6_1_5-01
4.2.3	Other findings	No indication of a treatment related effect on bodyweight.
4.3	**Overall result**	The Local Lymph Node Assay demonstrated that Cypermethrin cis:trans/40:60 does not have the potential to cause skin sensitisation at concentrations of up to 2.5% v/v in 80% v/v acetone in olive oil.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The Local Lymph Node Assay (LLNA) was used to assess the potential of cypermethrin cis:trans/40:60 to cause skin sensitisation in the mouse according to OECD guideline 429 (2002). Following a preliminary study, the test article was prepared for administration at 0.5%, 1.0% and 2.5% v/v in 80% v/v acetone in olive oil. Groups of five female CBA / Ca mice were subjected to topical applications of vehicle or of one of the test formulations to the outer aspect of the auditory pinnae once daily on Days 1, 2 and 3. In addition, a concentration of 25% v/v α-Hexylcinnamaldehyde in acetone / olive oil (4:1 v/v) was administered to a positive control group of five mice. On Day 6 a 20 µCi dose of tritiated ^{3}H-methyl thymidine was injected intravenously into each mouse. Five hours later the auricular lymph nodes were recovered from each animal. The pairs of nodes from each animal were pooled and suspensions of the cellular components of the lymph nodes were prepared in 5% w/v trichloroacetic acid and processed through a scintillation counter.
5.2	**Results and discussion**	Test results are expressed in terms of Stimulation Indices, the ratios of the mean scintillation counts obtained from the test groups relative to the corresponding mean scintillation count obtained from controls. The threshold level for the Stimulation Index to be considered a positive indicator of the potential to cause skin sensitisation is 3.0. The Local Lymph Node Assay demonstrated that Cypermethrin *cis:trans* 40:60 does not have the potential to cause skin sensitisation at concentrations of up to 2.5% v/v in 80% v/v acetone in olive oil.
5.3	**Conclusion**	The test article did not meet the criteria for classification as a sensitiser according to EU labelling regulations Commission Directive 2001/59/EC. No symbol and risk phrase are required.
5.3.1	Reliability	1
5.3.2	Deficiencies	None

Section A6.1.5 Skin sensitisation -

Annex Point IIA6.1.5 **Mouse Local Lymph Node Assay (LLNA)**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March, 2007.
Materials and Methods	The applicant's version is acceptable with the following amendment: 3.1.2.3 Stability: Stability of test substance in vehicle: data not shown.
Results and discussion	The applicant's version is adopted.
Conclusion	Cypemethrin is not found a dermal sensitizer as tested with the LLNA (conc. 2.5%, 1%, 0.5%). According to these test results, cypermethrin does not require classification.
Reliability	1
Acceptability	acceptable
Remarks	-
	COMMENTS FROM ...
Date	Give date of comments submitted
Materials and Methods	Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state
Results and discussion	Discuss if deviating from view of rapporteur member state
Conclusion	Discuss if deviating from view of rapporteur member state
Reliability	Discuss if deviating from view of rapporteur member state
Acceptability	Discuss if deviating from view of rapporteur member state
Remarks	

Table A6_1_5-01: Individual DPM's and Stimulation Index (SI)

Concentration (% v/v)	Group number	Animal number	DPM/ animal	Mean DPM/animal (Standard Deviation)	Stimulation Index (SI) [a]
Vehicle	1	293 294 295 296 297	704 943 1212 470 963	858 (± 282)	NA
0.5	2	298 299 300 301 302	520 57 208 413 311	302 (± 179)	0.35
1	3	303 304 305 306 307	85 280 60 561 988	395 (± 388)	0.46
2.5	4	308 325 326 327 328	447 557 860 457 400	544 (± 186)	0.63
Positive control	5	329 330 331 332 333	5792 3025 2050 4551 3594	3802 (± 1435)	4.43

[a] = Stimulation Index of 3.0 or greater indicates a positive result

NA = Not applicable

Section A6.12.1 Human Case Report

Annex Point IIA6.12.1 **Medical surveillance data on manufacturing plant personnel**

Official use only

		1 REFERENCE
1.1	**Reference**	Le Quesne, P.M., Maxwell, I.C. (1980); Transient Facial Sensory Symptoms following Exposure to Synthetic Pyrethroids: A Clinical and Electro-physiological Assessment; Neurotoxicology 2: 1-11 (CYP/T38) (published).
		2 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)
		3 MATERIALS AND METHODS
3.1	**Substance**	Volunteer subjects had been occupationally exposed to pyrethroids, including cypermethrin for up to five years.
3.2	**Persons exposed**	
3.2.1	Sex	Male and Female
3.2.2	Age/weight	20 to 52 years
3.2.3	Known Diseases	Not specified, however case histories were recorded during the study.
3.2.4	Number of persons	23
3.2.5	Other information	Control subjects: equal number of age and sex matched, no contact with pyrethroids.
3.3	**Exposure**	Occupational, therefore dermal and inhalation routes would be most likely
3.3.1	Reason of exposure	Occupational
3.3.2	Frequency of exposure	Multiple exposure, depending on occupational activity (2 workers had been involved in field trials, 8 in formulation and 13 in various types of laboratory work).
3.3.3	Overall time period of exposure	Degree of exposure was variable. 12 subjects were still working with pyrethroids and had done so for between 1 and 5 years, 8 had worked with pyrethroids in the past but not during the preceding year and 3 had only slight/occasional exposure or been in contact with pyrethroids for less than 1 year.
3.3.4	Duration of single exposure	Variable depending on occupation (see point 3.3.2)
3.3.5	Exposure concentration/dose	Variable depending on occupational activity.
3.3.6	Other information	Most subjects were exposed to several different pyrethroids including cypermethrin, permethrin, fenvalerate and fenpropathrin. Most has also been exposed to other chemicals in the past such as organophosphorus and carbamate insecticides.

Section A6.12.1 **Human Case Report**

Annex Point IIA6.12.1 **Medical surveillance data on manufacturing plant personnel**

3.4	**Examinations**	**Neurological examinations:** including tendon reflexes and sensory testing. Electrophysiological studies were performed on selected sensor and motor neurons in the legs and arms. No elevations were performed directly on the nerves and/or muscles of the face, where parasthesiae occurred. **Electrophysiological studies:** A Medelec MS6 electromyograph was used to estimate the maximum motor nerve conduction velocity in the forearm segment of the median nerve and the amplitude of the evoked muscle action potential recorded from the abductor pollicis brevis. Sensory nerve action potentials were recorded from the median and sural nerves.	
3.5	**Treatment**	No treatment was given during the study. Treatment of intoxication is supportive / symptomatic only.	
3.6	**Remarks**	-	
		4 RESULTS	
4.1	**Clinical Signs**	In the past, workers had typically experienced one or more episodes of abnormal facial sensation developing between 30 minutes and 3 hours after exposure. Symptoms persisted for 30 minutes to 8 hours.	X
4.2	**Results of examinations**	**Neurological Examinations:** No significant abnormality of either the motor or sensory nerves was found. Neurological examinations showed no muscle wasting or weakness. All tendon reflexes were present, although in 2 subjects this could only be elicited on reinforcement. Sensory testing was normal. According to records maintained by the Medical Advisor, no objective physical signs had ever been found in workers reporting with facial paraesthesiae. **Electrophysiological studies:** No statistical difference between the pyrethroid workers and the age-matched control subjects for either potential amplitude for the median or sural nerve or for conduction velocity for the median nerve.	
4.3	**Effectivity of medical treatment**	Not determined	
4.4	**Outcome**	-	
4.5	**Other**	-	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Clinical neurological examinations and electrophysiological studies have been conducted on 23 volunteer subjects. These persons had been occupationally exposed to synthetic pyrethroids, including cypermethrin, over periods from less than a year and up to five years, and had experienced the paresthesiae on more than one occasion. Electrophysiological studies were conducted on selected sensor and motor neurons in the legs and arms.	
5.2	**Results and discussion**	These studies failed to show any significant abnormality of either motor or sensory nerves.	

Section A6.12.1	**Human Case Report**
Annex Point IIA6.12.1	**Medical surveillance data on manufacturing plant personnel**

5.3	**Conclusion**	These studies failed to show any significant abnormality of either the motor or sensory nerves. It is concluded that symptoms such as facial sensations are most likely to be due to transient lowering of the threshold of sensory nerve fibres / endings following exposure of facial skin.

	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, 2007.
Materials and Methods	The applicant's version is acceptable..
Results and discussion	The applicant's version is adopted with the following amendment: 4.1 Clinical Signs: Sensations were described as 'tingling, burning, like coming in from the cold, nettle rash', but none complained of loss of sensation. The abnormal sensations were usually symmetrically distributed over the cheeks, particularly under the eyes and sometimes on the nose.
Conclusion	The applicant's version is adopted.
Remarks	
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.12.2 **Annex Point IIA.VI.6.9.2**	**Direct observation, e.g. clinical cases, poisoning incidents, if available**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [√]**	
Detailed justification:	No data is available. However one incident is discussed in the open literature (EHC no.82, WHO, 1989). A family who ate food cooked in 10% cypermethrin developed nausea and prolonged vomiting with colicky pain, tenesmus and diarrhoea within within minutes of ingestion. One male adult had convulsions and died due to respiratory paralysis, however symptoms were less severe in other members of the family. There is some doubt as to whether this incident was a cypermethrin intoxication.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
Date	**EVALUATION BY RAPPORTEUR MEMBER STATE** December, 2007	

Section A6.12.2 **Annex Point IIA.VI.6.9.2**	**Direct observation, e.g. clinical cases, poisoning incidents, if available**
Evaluation of applicant's justification	Despite their extensive world-wide use, there are **indeed few reports of human pyrethroid poisoning.** According to a review from **Bradberry et al. (2005)**, less than ten deaths have been reported from ingestion or following occupational exposure. A fatal case of human poisoning has been reported by **Vijvenberg and Van den Bercken (1990)**. A 45-year old man, who had accidently ingested more than 0.7g cypermethrin 10%, rapidly developed convulsions, passed into a coma, and died 3 hours later. The death of this man was ascribed to respiratory paralysis. **He et al. (1989)** reviewed 573 cases of acute pyrethroid poisoning reported in the Chinese medical literature between 1983-1988. Fourty-five cases of acute cypermethrin poisoning were detected (6 occupational, 39 accidental). Apart from the irritative symptoms of the skin and respiratory tract (or digestive tract in ingestive poisoning), acute pyrethroid poisoning was clinically characterised by abnormalities of nervous excitability. Initial symptoms were buring or itching sensation of the face or dizziness which usually developed at 4-6h after exposure. The skin symptoms could appear early after several minutes of spraying, followed by systemic symptoms as late as 48h after exposure. Symptoms and signs of acute poisoning: Half of the occupational exposed patients had abnormal facial sensations which could be exacerbated by sweating and washing with warm water. The systemic symptoms included dizziness, headache, nausea, anorexia, and fatigue. Weakness was found in 53.4% of the cases. Other symptoms included chest tightness, paresthesias, and in 11.9% of the cases palpitations, blurred vision, and increased sweating. Several patients showed low-grade fever and myopaia. Infrequently, seizures, pulmonary oedama, dyspnea, and cyanosis occurred. **Lessenger (1992)** reported 5 cases of poisoning by cypermethrin. After treating the air-conditioning system with cypermethrin, creating a fine vapour, employees were allowed to enter the treated building after 2 days. Already after 5 minutes the exposed employees experienced shortness of breath, dyspnea, wheezing, cough, congestion, nasal discharge, burning eyes, itching skin, nausea, and headaches. The employees could re-enter the building repeatedly and when they did, they experienced a return of their symptoms after turning on the air-conditioning system. Five employees were presented for examination. Shortness of breath persisted for over 2 weeks, and sore throat and sinus infections were still persistent 7 months post-exposure in one patient (non-smoker). Three other patients without previous pulmonary problems (of which 2 smokers) developed significant pulmonary dysfunction (still complaining of cough, congestion, and wheezing) 7 months post-exposure. **Das and Parajuli (2006)** recently reported a case of cypermethrin poisoning in Nepal. A 30-year old man was brought to the emergency department of the hospital with a history of vomiting, epigastiric pain, lacrimation, sweating and drooling after the ingestion of 50 ml of "Super-Cyprin" having a concentration of 25% cypermethrin (12.5 g). General examination revealed lips and buccal mucosa red and swollen, but vital and systemic examination were unremarkable. No fasciculation or tremor occurred and the liver function, renal function, Spo_2, haemogram, serum electrolytes and glucose tests were normal. A symptomatic treatment was given including treatment with activated charcoal, hyoscine butyl bromide for non-specific abdominal pain, and chlorpheniramine maleate for the increased salivation and red irritating eyes.
Conclusion	There is data available. However, indeed only few reports of human pyrethroid poisoning are made available in open literature.
Remarks	

Section A6.12.2 **Annex Point IIA.VI.6.9.2**	**Direct observation, e.g. clinical cases, poisoning incidents, if available**
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.12.3 **Annex Point IIA.VI.6.9.3**	**Health records, both from industry and any other available sources**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [√]**	
Detailed justification:	No data is available. However, it is well known within the pyrethroids industry that laboratory workers and field operators handling synthetic pyrethroids have noticed a transient 'tingling' sensation on the skin, particularly the face (EHC no.82, WHO, 1989). These effects are short lived, usually disappearing within a few hours after exposure.	
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	May, 2007.	
Evaluation of applicant's justification	The applicant's justification is accepted.	
Conclusion	The applicant's justification is accepted.	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A6.12.4 Epidemiological Study

Annex Point IIA6.12.4 **Cross sectional study occupational exposure**

Official use only

		1 REFERENCE
1.1	**Reference**	Chen , S., Zhang, S., He, F., Yao, P., Wu, Y., Sun, J., Liu, L., Li, Q. (1991); An epidemiological study on occupational acute pyrethroid poisoning in cotton farmers; British Journal of Industrial Medicine, 48: 77-81. (CYP/T164) (published).
1.2	**Data protection**	No
		2 GUIDELINES AND QUALITY ASSURANCE
		Not applicable
		3 MATERIALS AND METHODS
3.1	**Test material**	83.1% of the subjects were exposed to a number of pyrethroids including 2.5% deltamethrin, 20% fenvalerate EC and 10% cypermethrin. 16.9% had also been exposed to pyrethoids mixed with organophosphorus insecticides.
3.1.1	Lot/Batch number	Not available,
3.1.2	Specification	Not available
3.1.2.1	Description	Not available
3.1.2.2	Purity	Not available
3.1.2.3	Stability	Not available
3.2	**Type of study**	Cross-sectional survey conducted in 1987 and 1988
3.3	**Method of data collection**	Structured questionnaire and interview according to WHO field survey procedures
3.4	**Test Persons / Study Population**	
3.4.1	Selection criteria	Cotton farmers (spraymen) in 8 villages in China. Subjects not eligible were excluded such as children, those who had never used pesticides or who did not grow cotton. Also excluded were those who had not sprayed pyrethroids between June and August in 1987 and 1988 and who used pyrethroids mixed with organophosphates in 1988.
3.4.2	Number of test persons per group/cohort size	3113 subjects in total 38 subjects were selected for environmental and biological monitoring
3.4.3	Sex	2230 men, 883 women
3.4.4	Age	15-72 years (most being between 25 and 44)
3.4.5	Diseases	Not specified in report
3.4.6	Smoking status	Not specified in report
3.5	**Controls**	No
3.6	**Administration/ Exposure**	
3.6.1	Exposure Route	Dermal and secondary dermal, inhalation.
3.6.2	Exposure Situation	Occupational, during spraying.

Section A6.12.4		**Epidemiological Study**
Annex Point IIA6.12.4		**Cross sectional study occupational exposure**
3.6.3	Exposure concentration(s)	Most pyrethroids were diluted 1:4000 before use, the highest concentration for ULV application being 1:50.
3.6.4	Method(s) to determine exposure	Gas Chromatography was used to determine the concentrations of pyrethroids in the breathing zone. Exposure pads were used to assess dermal absorption onto clothes and skin. Urine samples were collected from 18 subjects.
3.6.5	Postexposure period	For urine analysis, samples were collected pre-exposure and at 3, 6, 9, 12 24, 48 and 72 hours after the beginning of the one day spraying.
3.7	**Examinations**	
3.7.1	Type of disease	Diagnosis of mild acute pyrethroid poisoning was made in individuals if they had abnormal facial sensations and significant systemic symptoms such as dizziness, headache, fatigue, nausea and loss of appetite, lethargy and muscular fasciculation.
3.8	**Further remarks**	None of the spraymen wore gloves or mask and most kept their upper extremities bare and wore sandals whilst spraying.
		4 RESULTS AND DISCUSSION
4.1	**Exposure**	Dermal exposure assessment (gauze pads) – 38 subjects
4.1.1.1	Average concentrations	Pyrethroids were detectable in 95.1% of samples (143 pads). Despite poor personal protection, the dermal contamination was less than 30% of their surface body.
4.2	**Number of cases for each disease / parameter under consideration**	In the cross sectional survey, adverse effects of pyrethroid exposure were found in 834 of the 3113 spray workers (26.8%). Symptoms manifested mainly as abnormal facial sensations, dizziness, headache, fatigue, nausea or loss of appetite. Only 10 subjects who developed significant systemic symptoms and had signs of lethargy or muscular fasciculation were diagnosed as having mild occupational acute pyrethroid poisoning with a prevalence of 0.31% in subjects exposed to pure pyrethroids and 0.38% in those exposed to pyrethroid/organophosphate mixtures.
4.3	**SMR (Standard mortality ratio), RR (relative risk), OR (Odds ratio)**	Not available
4.4	**Other Observations**	The survey on the knowledge and attitude of the workers to pyrethroid use showed that 69.8% were not aware of the toxicity of these compounds and that their personal protection was not satisfactory. Moreover skin contamination was seen in 92% of the subjects studied in 1987 and was mainly due to the preparation of pyrethroids by hand. And also the clearing of stoppages and leaks in the spraying equipment which 65% of workers cleared by hand or with their mouth.

Section A6.12.4	**Epidemiological Study**
Annex Point IIA6.12.4	**Cross sectional study occupational exposure**

		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Cross-sectional survey of pyrethroid spraymen from 8 villages in China conducted in 1987 and 1988. Subjects were exposed to a number of pyrethroids including deltamethrin, fenvalerate cypermethrin. Some had also been exposed to pyrethoids mixed with organophosphorus insecticides. Selected subjects were assessed for dermal exposure.
5.2	**Results and discussion**	834 (26.8%) of spraymen reported abnormal facial sensations, mainly burning and tingling which emerged as initial symptoms.
5.3	**Conclusion**	Dermal contamination is the main route of exposure to pyrethroids in cotton growers. Preventative measures (PPE) are to be recommended.
5.3.1	Reliability	2
5.3.2	Validity	Study was conducted on spray workers who wore no protective clothing and the spray operation did not conform to modern standards. However the study is useful in demonstrating the types of symptoms experienced and the major route of exposure.
5.3.3	Deficiencies	No, study was conducted according to WHO guidelines
5.4	**Other**	-

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, 2007.
Materials and Methods	The applicant's version is acceptable.
Results and discussion	The applicant's version is adopted.
Conclusion	The applicant's version is adopted.
Reliability	2
Acceptability	Acceptable.
Remarks	

Section A6.12.4 Epidemiological Study

Annex Point IIA6.12.4 **Cross sectional study occupational exposure**

	COMMENTS FROM ...
Date	Give date of comments submitted
Materials and Methods	Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state
Results and discussion	Discuss if deviating from view of rapporteur member state
Conclusion	Discuss if deviating from view of rapporteur member state
Reliability	Discuss if deviating from view of rapporteur member state
Acceptability	Discuss if deviating from view of rapporteur member state
Remarks	

Section A6.12.5 **Annex Point IIA.VI.6.9.5**	**Diagnosis of poisoning including specific signs of poisoning and clinical tests, if available**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [√]**	
Detailed justification:	No clinical data is available. However, it is well known within the pyrethroids industry that laboratory workers and field operators handling synthetic pyrethroids have noticed a transient 'tingling' sensation on the skin, particularly the face (EHC no.82, WHO, 1989). These effects are short lived, usually disappearing within a few hours after exposure. It can be expected that after accidental ingestion, symptoms will include nausea, vomiting and epigastric pain, dizziness, headaches and fatigue. In sever cases of exposure impaired consciousness, convulsions, coma and pulmonary oedema.	
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	December, 2007.	
Evaluation of applicant's justification	In humans, a variety of reversible symptoms have been reported (He et al., 1988, 1989). The initial symptoms with occupational intoxication are burning, itching, or tingling sensation of the face, or dizziness that usually developes 4 to 6 hours after exposure. The skin symptoms appear early after several minutes of spraying, followed by systemic symptoms as late as 48 h after exposure. After ingestion, the initial symptoms are mainly digestive such as epigastric pain, nausea, and vomiting, and develop within 10 minutes to 1 hour. Skin symptoms are not significant in patients with ingestive poisoning. The facial paresthesiae following direct skin contact with cypermethrin, are highly characteristic for pyrethroids, especially in the absence of any visible signs of skin irritation such as erythema, swelling, blistering, exudation, or desquamation. Indeed the signs of systemic poisoning by cypermethrin, following massive ingestion, appear to be non-specific. Acute intoxications by pyrethroids have been reported to lead to signs and symptoms such as dizziness, headache, nausea, anorexia, fatigue, gastrointestinal complaints, and fever. In severe cases, exposure results in impaired consciousness, muscular fasciculations, convulsions, coma, and pulmonary oedema. A blood cholinesterase test might proof useful to exclude organophosphate poisoning.	
Conclusion	The applicant's justification is acceptable with the amendment.	

Section A6.12.5 **Annex Point IIA.VI.6.9.5**	**Diagnosis of poisoning including specific signs of poisoning and clinical tests, if available**
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.12.6 **Annex Point IIA.VI.6.9.6**	**Sensitisation/allergenicity observations, if available**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [√]**	
Detailed justification:	No clinical data is available. However, it is well known within the pyrethroids industry that laboratory workers and field operators handling synthetic pyrethroids have noticed a transient 'tingling' sensation on the skin, particularly the face (EHC no.82, WHO, 1989). These effects are short lived, usually disappearing within a few hours after exposure. This parasthesia has been interpreted as being caused by repetitive firing of the nerve endings, with thresholds transiently lowered by the compound. These effects normally occur 30 minutes after skin exposure, lasting only a few hours and not persisting for more than one day.	
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	December, 2007.	

Section A6.12.6 **Annex Point IIA.VI.6.9.6**	**Sensitisation/allergenicity observations, if available**
Evaluation of applicant's justification	Only **few cases of contact dermatitis** due to the exposure to cypermethrin were reported. **Wagner (1994)** reported two cases. The first case was a 40 year old woman who's home was treated with cypermethrin. A spill occurred which contaminated the flooring of the bedroom and closet. The following day she developed a generalized urticarial eruption and on the second day post-application she was seen at the emergency because of increasing urticaria which had progressed to involve her eyelids. This case of contact allergic dermatitis, was confirmed with a patchtest with the formulated product. The second case was an applicator treating an area with a combination of cypermethrin andn cyfluthrin. Apart from the parasthesias of the exposed skin, this worker also developed an urticarial reaction. In a study investigating the role of pyrethroids with respect to irritation and their sensitisation potential, **Lisi (1992)** tested 7 pyrethroids in 230 subjects (54 patients with contact dermatitis, 176 with non-allergic skin disorders, 16 atopics) from different areas (males and females; agricultural workers, ex-agricultural workers, others) to establish the optimal test concentrations in the patch test and the frequencies of irritant and allergic reactions. Cypermethrin was tested at concentrations of 1%, 2%, 5% in petrolatum. The frequency of skin irritation and sensitisation was low. Positive irritant reactions were only seen in 2 subjects (2 fenvalerate). Positive allergic reactions were only seen in 3 subjects (2 fenvalerate, 1 cypermethrin), and the one to cypermethrin was not seen clinically relevant. Out of the results in this study, one can conclude that pyrethroids only have a very slight irritant and sensitizing potential.
Conclusion	In open literature, only few cases of contact dermatitis due to cypermethrin exposure are reported. Based on the human data made available in open literature, it can be concluded that cypermethrin has only a slight sensitizing potential.
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.12.7 **Annex Point IIA.VI.6.9.7**	**Specific treatment in case of accident or poisoning: first aid measures, antidotes and medical treatment, if known**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [√]**	
Detailed justification:	No specific study/clinical data. No specific antidote is known, treatment should be symptomatic and supportive. Ingestion: Do not induce vomiting. Rinse mouth but do not allow patient to swallow water. Seek medical advice immediately and show the container or label. Inhalation: Remove patient to fresh air and allow to rest. If breathing has stopped perform artificial respiration and seek medical advice immediately. Skin contact: Remove contaminated clothing immediately and wash skin with mild soap and water. If symptoms persist, consult a doctor. Eye contact: Rinse eye immediately with clean water for at least 10-15 minutes, holding eyelids open to ensure irrigation. Seek medical advice immediately.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
Date	**EVALUATION BY RAPPORTEUR MEMBER STATE** December, 2007.	

Section A6.12.7 **Annex Point IIA.VI.6.9.7**	**Specific treatment in case of accident or poisoning: first aid measures, antidotes and medical treatment, if known**
Evaluation of applicant's justification	Indeed, no specific antidote is known, treatment should be symptomatic and supportive. According to the review of Bradberry et al., 2005: Most patients exposed require only skin or eye decontamination and symptomatic and supportive measures. For paraesthesiae no specific treatment is generally required. However, topical application of vitamin E can reduce the severity of the skin reaction. Convulsions should be treated with anticonvulsants such as diazipan (5-10mg if seizures are prolonged). Induction of vomiting is not recommended following ingestion. However, gastric lavage should also be avoided, since solvents present in many formulations may increase the risk of aspiration pneumonia. Alternatively, the administration of active charcoal 50-100g to an adult may be considered of a potentially toxic amount has been ingested within 1 hour.
Conclusion	The applicant's justification is acceptable with the amendment.
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.12.8 **Annex Point IIA.VI.6.9.8**	**Prognosis following poisoning**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [√]**	
Detailed justification:	No clinical data is available. However, the effects of parasthesia are short lived, usually disappearing within a few hours after skin exposure and not lasting more than one day. These effects are an early indication of exposure and should be followed up with a review of work practises.	
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	May, 2007.	
Evaluation of applicant's justification	The applicant's justification is acceptable.	
Conclusion	The applicant's justification is acceptable.	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A 6.2 (01) Toxicokinetics/Metabolism

Annex Point IIA VI.6.2

ADE study in the rat

			Official use only
		1 REFERENCE	
1.1	**Reference**	Needham, D. (2006); [^{14}C]-Cypermethrin-cis:trans 40:60:- Absorption, Distribution and Excretion in the Rat; Covance Laboratories Limited, report no. 1669/029, 31 March 2006 (unpublished). Dates of experimental work: 14 April 2005 – 23 January 2006	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Chimac-Agriphar S.A.	
1.2.2			
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD Guideline 417, OPPTS 870.7485 (1998)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	Cypermethrin cis:trans/40:60	
3.1.1	Lot/Batch numbers	AS 175COV/05 (Cis Cypermethrin, non-radiolabelled) AS 176COV/05 (Trans Cypermethrin, non-radiolabelled)	
3.1.2	Specification	Deviating from the specification given in section 2 as follows	

Section A 6.2 (01) Toxicokinetics/Metabolism

Annex Point IIA VI.6.2

ADE study in the rat

3.1.2.1	Description	-
3.1.2.2	Purity	98.4 % w/w (Cis Cypermethrin, non-radiolabelled) 98.9 % w/w (Trans Cypermethrin, non-radiolabelled)
3.1.2.3	Stability	Stable
3.1.2.4	Radiolabelling	Radiolabelled cypermethrin was supplied as separate cis- and trans-cypermethrin labelled in either the cyclopropyl or phenyl ring: *Cis* [^{14}C-cyclopropyl]-cypermethrin Study number 04 BLY 115b Specific radioactivity 53 mCi/mmol (4.7 MBq/mg) Radiochemical purity 100%. *Trans* [^{14}C-cyclopropyl]-cypermethrin Study number 04 BLY 115b Specific radioactivity 53 mCi/mmol (4.7 MBq/mg) Radiochemical purity 100%. *Cis* [^{14}C-phenyl]-cypermethrin Study number 04 BLY 115b Specific radioactivity 55.4 mCi/mmol (4.9 MBq/mg) Radiochemical purity 100%. *Trans* [^{14}C-phenyl]-cypermethrin Study number 04 BLY 115b Specific radioactivity 55.4 mCi/mmol (4.9 MBq/mg) Radiochemical purity 100%.
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Sprague Dawley (Crl:CD®(SD) IGSBR)
3.2.3	Source	Charles River (UK) Ltd
3.2.4	Sex	Males and Females
3.2.5	Age/weight at study initiation	Males: 188 – 301 g Females: 180 – 247 g
3.2.6	Number of animals per group	4 males, 4 females (excretion balance studies) 12 males, 12 females (tissue distribution study) See Table A6.2_01-1
3.2.7	Control animals	No
3.3	**Administration/ Exposure**	Oral
3.3.1	Type	Gavage
3.3.2	Concentration of test substance	See Table A6.2_01-1

Section A 6.2 (01) Toxicokinetics/Metabolism

Annex Point IIA VI.6.2

ADE study in the rat

3.3.3	Preparation of test substance	Appropriate amounts of the non-radiolabelled *cis* and *trans* isomers were weighed into a formulation vessel. Appropriate volumes of the *cis* and *trans* isomers of [^{14}C]-cypermethrin were then transferred to the same formulation vessel which was agitated to co-dissolve the non-radiolabelled test substance. The solvent was removed under a stream of nitrogen and an appropriate volume of corn oil was then added to the test substances, which were dissolved using sonication or mixing.
3.3.4	Dose volume	5 ml/kg
3.3.5	Dose administration	Single doses were administered in the balance study. In the distribution study, doses were administered daily for up to 9 days
3.3.6	Sampling time	See Table A6.2_01-1 At each collection timepoint, cage debris was removed and the cages washed with water and then a methanol wash. Cage debris and washings were pooled separately for each animal
3.3.7	Tissue analysis	<u>Balance studies</u> Following the final sample collection, the animals were exsanguinated by cardiac puncture under anaesthesia and weighed blood samples taken into two heparin-lined tubes, one of which was used to prepare plasma. The following tissues were also taken and the residual carcasses retained for analysis: Adrenals, bone, brain, fat, GI tract (+ contents) heart, kidneys, liver, lungs, muscle (quadriceps), ovaries and uterus (females only), skin, spleen, testis (males only). <u>Distribution study</u> Animals were killed by cold shock in a mixture of hexane and solid carbon dioxide following deep anaesthesia. Carcasses were retained in the freezing mixture for at least 30 mins and were then stored frozen (-20°C) before being prepared for QWBA. Blood samples were also taken prior to terminal sacrifice in order to prepare plasma.
3.3.8	Treatment of samples	Carcasses were digested in potassium hydroxide (40% solution in methanol) under reflux. Digests were neutralised prior to LSC analysis. Blood samples were incubated with solubilising agent. Liquid scintillant was then added and the samples left to dark-adapt prior to LSC analysis. Faeces, cage debris and tissues were similarly treated with solubilising agent and left to incubate before the addition of liquid scintillant. <u>Quantitative Whole body Autoradiograpy (QWBA)</u> Legs, whiskers and tail were trimmed off and each frozen carcass was set in a block of aqueous 2% (w/v) carboxymethylcellulose. Sagittal sections (nominal thickness 30 µm) were obtained at a minimum of 5 levels through the carcass using a cryomicrotome. These levels included, but were not limited to, the following tissues: exorbital lachrymal gland (males) or ovary (females), intra-orbital lachrymal gland, Harderian gland, adrenal gland, thyroid, brain and spinal cord. The sections were mounted, freeze-dried and placed in contact with FUJI imaging plates. [^{14}C]-Blood standards of appropriate activity (also sectioned at a nominal thickness of 30 µm) were placed in contact with all imaging plates and exposed for 7 days in a copper lined lead exposure box. After exposure, the imaging plates were processed using a FUJI FLA 5000

Section A 6.2 (01) **Toxicokinetics/Metabolism**

Annex Point IIA VI.6.2 **ADE study in the rat**

radioluminography system. The carbon-14 blood standards included with each autoradiogram were used to construct calibration lines over a range of radioactivity concentrations.

4 RESULTS AND DISCUSSION

4.1 Excretion Balance study

Low Dose (3mg/kg bw)

The rats of Group A received a single oral dose of [^{14}C-cyclopropyl]-cypermethrin at a mean level of 3.417 mg/kg bodyweight. Analysis of the levels of radioactivity in the urine and faeces showed that the excretion was virtually complete by 72 h after dosing. The mean overall recovery was 102.9 ± 2.6% of the dose. The excretion of radioactivity was split equally between the urine (47.8% of the dose in males, 52.9% in females) and faeces (50.2% in males and 43.4% in females). There was no significant elimination of [^{14}C]-carbon dioxide in the expired air (<0.3% of the dose). The minimum absorption, as measured by the radioactivity excreted in the urine plus cage washes and debris, was 52.8% of the dose in the males and 57.6% in the females. The residual carcass contained <0.7% of the dose showing that elimination of the radioactive dose was complete.

The rats of Group C received a single oral dose of [^{14}C-phenyl]-cypermethrin at a mean level of 3.051 mg/kg bodyweight. As with the [^{14}C-cyclopropyl]-cypermethrin, analysis of the levels of radioactivity in the urine and faeces showed that the excretion was virtually complete by 72 h after dosing. The mean overall recovery of radioactivity was 101.4 ± 5.3%. The main route of excretion was *via* the faeces (48.5 and 59.8% of the dosed radioactivity in males and females respectively) with the urine containing a further 47.5% of the dose in the males and 40.8% in the females, though there was significant inter-individual variation. There was no significant elimination of [^{14}C]-carbon dioxide in the expired air (below the LOQ). The minimum absorption, as measured by the radioactivity excreted in the urine plus cage washes and debris, was 51.3% of the dose in the males and 43.6% in the females. The residual carcass contained 0.4 - 0.6% of the dose showing that elimination of the radioactive dose was essentially complete.

High Dose (50mg/kg bw)

The rats of Group B received a single oral dose of [^{14}C-cyclopropyl]-cypermethrin at a mean level of 50.186 mg/kg bodyweight. As with the lower dose, analysis of the levels of radioactivity in the urine and faeces showed that the excretion was virtually complete by 72 hours after dosing. The mean overall recovery was 106.0 ± 5.7% of the dose. The mean recovery values showed a slight sex difference in the excretion of the radioactivity with more of the dose being excreted in the urine of the females, though, there were significant inter-individual variations in the route of excretion. However, the main route of excretion was *via* the faeces (78.6 and 60.7% of the dosed radioactivity in males and females respectively), the urine contained a further 27.2% of the dose in the males and 36.9% in the females. There was no significant elimination of [^{14}C]-carbon dioxide in the expired air (<0.2% of the dose). The minimum absorption, as measured by the radioactivity excreted in the urine plus cage washes and debris, had fallen at the high dose to 28.7% of the dose in the males and 42.5% in the females. The

Section A 6.2 (01) **Toxicokinetics/Metabolism**

Annex Point IIA VI.6.2 **ADE study in the rat**

residual carcass contained approximately 0.4% of the dose showing that elimination of the radioactive dose was complete.

The rats of Group D received a single oral dose of [^{14}C-phenyl]-cypermethrin at a mean level of 48.749 mg/kg bodyweight. Analysis of the levels of radioactivity in the urine and faeces showed that the excretion was virtually complete by 72 h after dosing . The mean overall recovery was 109.7 ± 4.9% of the dose. The main route of excretion was *via* the faeces (80.0 and 68.3% of the dosed radioactivity in males and females respectively), with the urine containing a further 29.1% of the dose in the males and 34.8% in the females. There was no significant elimination of [^{14}C]-carbon dioxide in the expired air (below the limit of quantification). The minimum absorption, as measured by the radioactivity excreted in the urine plus cage washes and debris, was 31.5% of the dose in the males and 38.4% in the females. The residual carcass contained 0.5% of the dose showing that elimination of the radioactive dose was essentially complete.

See Table A6_2_01-2

4.2 Radioactivity in blood samples

At necropsy, 144 h after dosing, the mean concentration of radioactivity in the plasma of the low dose group animals was 2.56 ng equivalents/g for males and 0.81 ng equivalents/g for the females dosed with the cyclopropyl label, and below the limit of quantification in males and females dosed with the phenyl label.

In the high dose group, the mean concentration of radioactivity in the plasma was 12.6 and 11.0 ng equivalents/g in males and females dosed with the cyclopropyl label and was below the limit of quantification in all animals dosed with the phenyl label.

See Table A6_2_01-3

4.3 Radioactivity in tissue samples

The levels of radioactivity measured in the tissues generally reflected the lipophilic nature of cypermethrin with the highest levels being found in the fat in all dose groups. These levels were approximately 6 times higher than any other tissue in the case of the male rats. In female rats, the ovaries generally contained the next highest concentrations of radioactivity, though these were approximately 3 times lower than concentrations in the fat.

The high dose rate was 15-17 times greater than the low dose, and the concentration of radioactivity in the tissues did not automatically increase in direct proportion to the dose level. The concentration of radioactivity in the tissues in rats receiving [^{14}C-cyclopropyl]-cypermethrin, were 7-9 times greater at the high dose than at the low dose for the fat, liver and kidneys and 23 times greater for the adrenals. In the females, the concentrations of radioactivity in the tissues were 6-10 times greater at the high dose for the liver and adrenals, and 14-17 times for the fat, kidney, and ovaries.

When the rats were dosed with [^{14}C-phenyl]-cypermethrin, the concentration in the tissues of male animals were 9-14 times higher for the fat, liver and kidney, and 20 times higher for the adrenal. In the

Section A 6.2 (01) **Toxicokinetics/Metabolism**

Annex Point IIA VI.6.2 **ADE study in the rat**

females, the concentrations of radioactivity was 15 times higher for the liver, 6 times higher for the adrenal, and 15-21 times higher for the kidney, fat and ovaries.

4.4 QWBA – Tissue distribution study

The highest levels of radioactivity were found in the fat (peri-renal, inguinal and subcutaneous) at all timepoints. Residues were rapidly cleared from the body once dosing had ceased.

In males, the levels in the plasma 24 h after nine doses (565.5 ng equivalents/g) were twice those seen 24 h after a single oral dose. The highest increases (>10-fold) in the concentration of radioactivity were measured in the inguinal and peri-renal fat. In these tissues, the concentrations of residues rose from 91.8 to 1009 ng equivalents/g in the case of the inguinal fat and from 197.5 to 1966 ng equivalents/g in the case of the peri-renal fat. The lowest levels of radioactivity were seen in the brain (<9 ng equivalents/g) and spinal cord (<36 ng equivalents/g).

In female rats, the levels of radioactivity in the plasma were approximately 20% higher on Day 10 (698.2 ng equivalents/g) than on Day 2 (579.5 ng equivalents/g). The levels in the inguinal and peri-renal fat rose by 6-7 times those seen on Day 2, the concentrations of residues rising from 204 to 1196 ng equivalents/g in the case of the inguinal fat and from 295 to 2179 ng equivalents/g in the case of the peri-renal fat. The lowest levels of radioactivity were seen in the brain and spinal cord (<21 ng equivalents/g).

The radioactivity in the tissues was rapidly cleared, and by Day 16, 7 days after the last dose, many of the tissues contained levels of radioactivity that had fallen below the limit of detection. The concentrations of radioactivity in the fats had fallen by 2-6 times when compared to the levels on Day 10 whilst the levels in the plasma had fallen by approximately 30 times.

See Tables A6_2_01-4 and A6_2_01-5

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The absorption, distribution and excretion of cypermethrin was investigated according to OECD guideline 417. Male and female rats (Sprague Dawley (Crl:CD ® (SD) IGSBR) strain) were given a single oral dose of 3 or 50 mg [^{14}C]-cypermethrin labelled in either the cyclopropyl or phenyl ring and the rates and routes of excretion of the radioactivity were determined. A separate group of rats received up to 9 daily oral doses of 3 mg [^{14}C-phenyl]-cypermethrin/kg bodyweight and the concentration of radioactivity was determined in the tissues at 24 h after 1, 7 and 9 doses and at 7 days after 9 doses.

5.2 Results and discussion

At the higher dose level, faecal excretion was the major route of elimination accounting for 79 and 61% when [^{14}C-cyclopropyl]-cypermethrin was dosed and 80 and 68% when [^{14}C-phenyl]-cypermethrin was dosed. In each case, the higher excretion level was seen in the male rats. The observed increase in faecal elimination suggests that the absorption process was being saturated at

X

Section A 6.2 (01) **Toxicokinetics/Metabolism**

Annex Point IIA VI.6.2 **ADE study in the rat**

the higher dose level.

At the low dose, a minimum of 43.6 to 57.6% of the dose was absorbed by the rats, as measured by the total radioactivity in urine and cage washes. At the high dose, a minimum of 28.7 to 31.5% of the dose was absorbed by the male rats and 38.4 to 42.5% in the case of the females.

Only trace amounts of radioactivity were measured in the expired carbon dioxide confirming that the positions of radiolabel were metabolically stable in the rat.

At necropsy, 144 h after dosing, the levels of radioactivity measured in the tissues generally reflected the lipophilic nature of cypermethrin with the highest levels being found in the fat in all dose groups. These levels were approximately 6 times higher than any other tissue in the case of the male rats. In female rats, the ovaries generally contained the next highest concentrations of radioactivity, approximately 3 times lower than those seen in the fat.

The high dose rate was 15-17 times greater than the low dose, and the concentration of radioactivity in the tissues did not automatically increase in direct proportion to the dose level. The concentration of radioactivity in the tissues in rats receiving [^{14}C-cyclopropyl] -cypermethrin, were 7-9 times greater at the high dose than at the low dose for the fat, liver and kidneys and 23 times greater for the adrenals. In the females, the concentrations of radioactivity in the tissues was 6-10 times greater at the high dose for the liver and adrenals, and 14-17 times for the fat, kidney, and ovaries.

When the rats were dosed with [^{14}C-phenyl]-cypermethrin, the concentration in the tissues of male animals were 9-14 times higher for the fat, liver and kidney, and 20 times higher for the adrenal. In the females, the concentrations of radioactivity was 15 times higher for the liver, 6 times higher for theadrenal, and 15-21 times higher for the kidney, fat and ovaries.

Following repeated daily oral administration of [^{14}C-phenyl]-cypermethrin at a dose level of 3 mg/kg for up to 9 days, the levels of radioactivity in the tissues increased with the number of doses received. In males, the levels in the plasma 24 h after 9 doses were twice those seen 24 h after a single oral dose. The highest increase in the concentration of radioactivity were measured in the inguinal and peri-renal fat, and the spleen (>10-fold). In female rats, the levels of radioactivity in the plasma were approximately 20% higher on Day 10 than on Day 2 and the levels in the inguinal and peri-renal fat rose by 6-7 times those seen on Day 2.

Following the cessation of daily dosing, the radioactivity in the tissues was rapidly cleared, and by Day 16, 7 days after the last dose, many of the tissues contained levels of radioactivity that had fallen below the limit of quantification. The concentrations of radioactivity in the fat had fallen by 2-7 times when compared to the levels on Day 10 whilst the levels in the plasma had fallen by approximately 30 times.

5.3 Conclusion

Excretion of radioactivity was virtually complete by 72 h following a single oral dose of [^{14}C-cyclopropyl]- or [^{14}C-phenyl]-cypermethrin at a

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dose rate of 3 or 50 mg/kg bodyweight. Urinary and faecal excretion were similar at the low dose for both radiolabels, but at the higher dose level faecal excretion predominated, especially in the males. This suggests that the absorption of cypermethrin was being saturated at the high dose rate. As minimum of 43.6-57.6% of the dose was absorbed at the low dose level. At the high dose level, a minimum of 28.7 to 31.5% of the dose was adsorbed in male rats and 38.4 to 42.7% in the case of the females. At 144 h after dosing, the highest residues were found in the fat for all dose groups.

Following repeated daily oral dosing of 3 mg [^{14}C-phenyl]-cypermethrin, the levels of radioactivity rose by 6-7 times in the female rats, and by >10 times in the males. The lowest levels of radioactivity were seen in the brain and spinal cord. The tissue residues were rapidly cleared following the cessation of dosing, with the levels of radioactivity in the plasma falling by approximately 30 times over a 7 day period, and the levels in the fat falling by 2-7 times.

5.3.1 Reliability 1

5.3.2 Deficiencies None

Evaluation by Competent Authorities

Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

EVALUATION BY RAPPORTEUR MEMBER STATE

Date March, 2007.

Materials and Methods The applicant's version is acceptable with the following amendments:

Deviations protocol:

Because of the nature of the formulation, a solution of cypermethrin in corn oil, a trial formulation to assess homogenicity, stability, and radioactivity concentration was not performed prior to the preparation of the formulations for dose administration. The formulation prepared for dose group A was subsequently used to determine homogeniceity and stability at 4 and 11 days after preparation.

A number of rats were above the weight range (180-220g).

The number, quantity and identity of radiolabelled metabolites in urine, faeces, and bile and a proposed metabolic pathway were not determined in the study.

Results and discussion The applicant's version is acceptable with the following amendments:

Following a single oral dose of either 3 or 50 mg cypermethrin/kg bw to the rat, the excretion of radioactivity was virtually complete within 72h. There was little difference between the rates and routes of excretion of either of the radiolabelled forms of cypermethrin though there was significant inter-individual variations in the data. At the low dose urinary and faecal excretion were comparable when [^{14}C-cyclopropyl]-cypermethrin was dosed, but slightly higher urinary excretion was seen in the females dosed with [^{14}C-phenyl]-cypermethrin.

Added in table A6_2_01-1: actual dose rates

Conclusion The applicant's version is adopted.

Reliability 1

Section A 6.2 (01)	**Toxicokinetics/Metabolism**
Annex Point IIA VI.6.2	**ADE study in the rat**
Acceptability	Acceptable
Remarks	-
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6_2_01-1: Treatment schedule and dose rates

Dose Group	Frequency of dose	Label	Study type	Dose level		Act. dose rate	Number of animals		Sampling (hours after dose administration
				mg/kg	MBq/kg	mg/kg	M	F	
A	Single	cyclopropyl	Ex Bal	3	5	3.42	4	4	Urine: 6, 12, 24, 48, 72, 96, 120, 144 Faeces: 24, 48, 72, 96, 120, 144 Expired air: 24, 48
B	Single	cyclopropyl	Ex Bal	50	5	50.19	4	4	Urine: 6, 12, 24, 48, 72, 96, 120, 144 Faeces: 24, 48, 72, 96, 120, 144 Expired air: 24, 48
C	Single	phenyl	Ex Bal	3	2	3.05	4	4	Urine: 6, 12, 24, 48, 72, 96, 120, 144 Faeces: 24, 48, 72, 96, 120, 144 Expired air: 24, 48
D	Single	phenyl	Ex Bal	50	2	48.75	4	4	Urine: 6, 12, 24, 48, 72, 96, 120, 144 Faeces: 24, 48, 72, 96, 120, 144 Expired air: 24, 48
E	Repeated	phenyl	TD	3	2	3.01	12	12	3 M + 3 F sacreficed 24 h after 1, 7 and 9 doses and 7 days after the last dose.

Ex. Bal - Excretion balance study
TD - Tissue distribution study

Table A6_2_01-2: Overall recovery (mean % of administered dose)– Ex. Bal. study

Excreta	Time-point (h)	Low Dose (3 mg/kg bw)				High Dose (50 mg/kg bw)			
		[^{14}C]-cyclopropyl (Group A)		[^{14}C]-phenyl (Group C)		[^{14}C]-cyclopropyl (Group B)		[^{14}C]-phenyl (group D)	
		Males	Females	Males	Females	Males	Females	Males	Females
Urine	6	1.163	2.676	5.686	4.203	3.242	1.071	5.265	3.873
Urine	12	15.640	23.301	15.771	14.768	5.415	0.152	8.983	9.404
Urine	24	18.396	18.897	20.444	14.215	10.641	0.112	11.306	14.554
Urine	48	9.674	5.807	4.474	6.623	5.731	0.836	2.818	6.079
Urine	72	1.894	0.983	0.685	0.581	1.393	1.023	0.393	0.494
Urine	96	0.675	0.850	0.242	0.220	0.468	0.283	0.183	0.215
Urine	120	0.281	0.293	0.131	0.134	0.162	0.209	0.105	0.121
Urine	144	0.125	0.130	0.108	0.077	0.100	0.108	0.064	0.079
	Subtotal	47.846	52.935	47.541	40.822	27.150	36.883	29.115	34.816

Faeces	24	41.522	39.353	39.000	46.737	72.486	51.200	72.090	62.094
Faeces	48	7.135	3.305	6.269	9.980	4.849	7.366	6.354	5.808
Faeces	72	1.019	0.444	1.789	2.218	0.803	1.071	1.380	0.225
Faeces	96	0.318	0.154	1.312	0.161	0.291	0.152	0.241	0.094
Faeces	120	0.148	0.085	0.101	0.694	0.130	0.112	0.044	0.045
Faeces	144	0.095	0.050	0.042	0.016	0.070	0.836	0.020	0.011
	Subtotal	50.236	43.389	48.512	59.869	78.629	60.737	80.127	68.275
Cage Wash + Debris		4.992	4.624	3.717	2.773	1.591	5.660	2.352	3.582
CO2 Traps		0.255	0.151	BLQ	BLQ	0.121	0.164	BLQ	BLQ
Carcass	144	0.680	0.523	0.584	0.338	0.462	0.410	0.469	0.417
G.I. Tract +Contents	144	0.133	0.115	0.027	BLQ	0.068	0.056	0.083	0.110
	Subtotal	0.813	0.637	0.611	0.338	0.530	0.466	0.553	0.527
Overall recovery		104.143	101.735	100.381	102.487	108.023	103.91	112.145	107.198

BLQ = Below Limit of Quantification (DPM in sample below twice background)

Table A6_2_01-3: Mean concentration of radioactivity in tissues (ng equivalents/g tissue) – Ex. Bal

Tissue Sample	**Low Dose (3 mg/kg bw)**				**High Dose (50 mg/kg bw)**			
	[^{14}C]-cyclopropyl (Group A)		**[^{14}C]-phenyl (Group C)**		**[^{14}C]-cyclopropyl (Group B)**		**[^{14}C]-phenyl (Group D)**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Carcass	26.146	21.010	19.626	12.971	266.745	253.66	265.985	255.750
Skin	41.127	29.800	BLQ	BLQ	BLQ	315.963	329.725	BLQ
Plasma	2.564	0.808	BLQ	BLQ	12.597	10.961	BLQ	BLQ
Blood	2.392	1.500	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Brain	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Fat	321.490	227.665	254.38	232.78	2331.9	3184.1	3129.0	4895.35
Heart	2.207	1.640	BLQ	BLQ	15.314	BLQ	BLQ	BLQ
Lung	10.700	11.271	BLQ	BLQ	106.492	99.992	BLQ	BLQ
Spleen	5.115	6.092	BLQ	BLQ	23.323	45.895	BLQ	BLQ
Liver	48.021	21.856	14.411	11.651	408.84	226.81	126.660	170.515
Kidney	15.422	13.134	17.900	20.547	135.68	220.55	243.56	385.50
Testes	3.649	-	3.316	-	BLQ	-	BLQ	-
Ovaries	-	69.490	-	66.516	-	1178.1	-	1388.3
Adrenals	16.713	47.593	16.343	32.668	388.205	292.010	321.045	185.730
uterus	-	18.579	-	40.122	-	364.535	-	1024.5
Muscle (Quadriceps)	6.545	1.247	BLQ	1.931	17.505	20.613	BLQ	BLQ
Bone	5.450	3.760	1.455	BLQ	53.250	69.641	46.660	BLQ

BLQ = Below Limit of Quantification (DPM in sample below twice background)

Table A6_2_01-4: Mean concentration of radioactivity in tissues -Repeated dose study, male rats

	Mean Concentration of [^{14}C]-Cypermethrin residues (ng equivalents/g tissue)			
Number of doses given	**1**	**7**	**9**	**9**
Kill time	**Day 2**	**Day 8**	**Day 10**	**Day 16**
Plasma	292.9	432.5	565.5	17.90
Blood	182.2	241.7	315.4	BLQ
Aorta	193.7	292.7	540.6	BLQ
Mandibular lymph nodes	53.57	131.6	323.6	91.97
Kidney cortex	231.4	545.9	652.5	78.71
Kidney medulla	137.6	361.0	430.3	BLQ
Liver	180.7	455.8	693.9	88.29
Brain	BLQ	7.05	8.18	BLQ
Pineal body	92.67	BLQ	167.16	BLQ
Spinal cord	BLQ	21.04	35.83	BLQ
Adrenal	129.7	753.1	686.7	291.0
Pituitary	BLQ	293.2	58.83	BLQ
Thymus	23.50	38.56	48.11	BLQ
Thyroid	104.2	184.4	427.2	45.10
Exorbital lachrymal gland	45.51	80.49	109.1	BLQ
Harderian gland	93.52	255.3	224.2	10.31
Intra-orbital lachrymal gland	40.75	149.2	157.2	53.49
Salivary glands	35.73	62.17	100.3	19.42
Brown fat	568.8	1565	1936	321.1
Inguinal fat	91.81	953.4	1009	581.0
Peri-renal fat	197.5	1319	1966	717.7
Subcutaneous fat	86.63	381.9	351.6	73.48
Bulbo-urethral gland	43.18	73.71	116.10	BLQ
Epididymis	43.05	209.5	102.2	193.8
Preputial gland	82.20	431.8	635.4	367.4
Prostate	56.29	119.3	170.2	190.4
Seminal vesicles	48.82	31.99	366.6	204.3
Testis	41.87	59.87	65.82	8.18
Muscle	21.42	30.50	36.82	BLQ
Myocardium	59.39	102.0	127.5	BLQ
Tongue	53.84	81.50	128.7	BLQ
Skin	79.05	224.5	208.7	99.74
Uveal tract	76.22	76.27	132.9	BLQ
Bone marrow	32.79	76.27	62.71	29.98
Lung	136.4	195.4	260.0	7.82
Pancreas	43.21	98.76	72.81	20.56
Spleen	36.80	66.18	369.2	12.43
Tooth pulp	51.06	91.33	164.1	BLQ
Nasal mucosa	104.3	141.8	201.2	28.12
Oesaphagus wall	71.57	170.7	130.1	BLQ
Stomach mucosa	52.77	130.3	184.6	BLQ
Small intestine mucosa	270.8	650.6	471.5	BLQ
Caecum mucosa	167.8	321.3	1232	126.8
Large intestine mucosa	488.2	1202	2490	52.51
Rectum mucosa	111.4	599.8	584.6	BLQ
Limit of Quantification	23.50	22.11	21.75	22.01

BLQ - Tissue measurement below lower limit of quantification

NA - Not Available

Table A6_2_01-5: Mean concentration of radioactivity in tissues -Repeated dose study, female rats

	Mean Concentration of [^{14}C]-Cypermethrin residues (ng equivalents/g tissue)			
Number of doses given	**1**	**7**	**9**	**9**
Kill time	**Day 2**	**Day 8**	**Day 10**	**Day 16**
Plasma	579.5	548.4	698.2	24.40
Blood	381.1	344.0	452.9	22.41
Aorta	416.7	462.3	493.5	BLQ
Mandibular lymph nodes	117.3	145.2	154.8	52.88
Kidney cortex	439.6	656.4	926.1	127.2
Kidney medulla	276.9	167.7	238.5	26.22
Liver	651.6	882.9	991.8	103.3
Brain	7.744	9.187	15.54	BLQ
Pineal body	131.5	127.1	96.67	BLQ
Spinal cord	8.279	8.706	20.14	BLQ
Adrenal	220.3	342.9	784.1	358.9
Pituitary	78.67	NA	77.07	19.12
Thymus	36.21	37.97	384.3	BLQ
Thyroid	149.1	139.9	285.2	7.424
Harderian gland	78.99	138.1	193.9	23.93
Intra-orbital lachrymal gland	107.1	127.1	189.3	57.42
Salivary glands	72.32	91.06	152.6	8.599
Brown fat	882.9	1176	1831	280.9
Inguinal fat	203.7	1032	1196	347.2
Peri-renal fat	294.6	1250	2179	705.5
Subcutaneous fat	156.4	385.6	385.1	151.4
Clitoris	140.0	242.7	346.1	64.89
Ovary	247.8	837.5	1042*	715.2*
Uterus	233.1	549.7	943.8	BLQ
Muscle	26.44	23.39	34.66	BLQ
Myocardium	121.2	102.6	161.4	8.439
Tongue	102.5	109.4	150.3	BLQ
Skin	145.5	626.5	619.6	339.7
Uveal tract	166.2	166.6	229.1	BLQ
Bone marrow	92.24	212.0	198.6	111.9
Lung	255.3	258.0	322.1*	30.60
Pancreas	81.24	88.45	201.3	15.65
Spleen	48.60	NA	100.6	BLQ
Tooth pulp	163.2	148.1	236.6	BLQ
Nasal mucosa	168.5	127.9	370.9	51.75
Oesaphagus wall	103.3	210.8	275.4	BLQ
Stomach mucosa	209.4	157.3	341.3	BLQ
Small intestine mucosa	197.1	190.6	1473	BLQ
Caecum mucosa	556.0	562.9	591.8	BLQ
Large intestine mucosa	731.2	733.3	1344	BLQ
Rectum mucosa	1056	195.5	1764	BLQ
Limit of Quantification	23.23	25.96	22.11	22.11

BLQ – Tissue measurement below lower limit of quantification

NA – Not Available

* - Measurement affected by high levels of radioactivity in surrounding fat or tissue

Section A6.2 (02) Percutaneous absorption

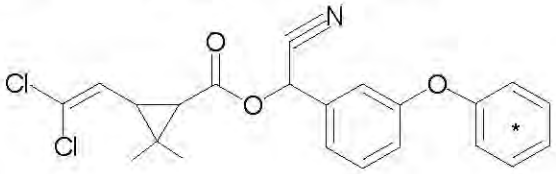
Annex Point IIA6.2 **In-vitro dermal absorption in human skin**

			Official use only
		1 REFERENCE	
1.1	**Reference**	Hardwick T, (2005); [^{14}C]-Cypermethrin cis:trans 40:60 – Rates of penetration through human skin using a static cell *in-vitro* system; Covance Laboratories Ltd, Study no. 1669/028, 10 January 2006 (unpublished). Dates of experimental work: 6 July 2005 – 8 August 2005	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Chimac-Agriphar s.a.	
1.2.2			
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes. Dermal delivery and percutaneous absorption: *in-vitro* method. OECD guideline 428 (adopted 13 April 2004).	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	[^{14}C]-Cypermethrin cis:trans/40:60 administered in a 'blank' formulation vehicle consisting of solvent and emulsifiers which make up the standard Emulsifiable Concentrate. The EC formulation was used in order to give a worst case realistic operator exposure.	
3.1.1	Lot/Batch number	[^{14}C]-Cypermethrin *cis* - 04 BLY 115b [^{14}C]-Cypermethrin *trans* - 04 BLY 115b Cypermethrin *cis* – AS 175COV/05 Cypermethrin *trans* – AS 176COV/05 Formulation vehicle - BA0625/05 (code CA 711348)	
3.1.2	Specification	Deviating from specification given in section 2 as follows	

Section A6.2 (02) Percutaneous absorption

Annex Point IIA6.2 **In-vitro dermal absorption in human skin**

3.1.2.1	Description	Pale yellow viscous liquid / semi-solid
3.1.2.2	Purity	[14C-phenoxy]-Cypermethrin *cis* and *trans* – specific radioactivity 55.4 mCi/mmol (4.9 MBq/mg), radiochemical purity 100%. Non-radiolabelled Cypermethrin *trans* – chemical purity 98.9% Non-radiolabelled Cypermethrin *cis* – chemical purity 98.4%
3.1.2.3	Stability	Stable
3.1.2.4	Radiolabelling	^{14}C labelled Cypermethrin cis:trans/40:60



*Position of radiolabelled isotope

3.2	**Test System**	
3.2.1	Species	Man (human skin samples)
3.2.2	Source	Human Caucasian skin was obtained from Biopredic International. Samples arrived frozen, on solid carbon dioxide. Only those tissues in which the epidermal layer was intact at excision and where the donor had not received medical treatment that could have compromised the integrity of the study, if known, were used.
3.2.3	Number of donors per group	Four donors were used, with each donor providing at least 3 replicates in each group.
3.2.4	Control animals	No
3.3	**Administration/ Exposure**	Dermal (skin membrane)
3.3.1	Preparation of test system	Ten diffusion cells were prepared for each of the two dose levels. The skin preparation was drawn over the receptor chamber of a glass diffusion cell and clamped between the donor and receptor chambers. Excess skin was trimmed as appropriate. The exposed surface area of the epidermis, as demarcated by the donor chamber, was 1.77 cm^2 (1.5 cm diameter). The volume of the receptor chamber was approximately 5 ml. Due to the low solubility of Cypermethrin cis:trans 40:60, the receptor fluid selected was ethanol/water (1:1, v/v). A membrane integrity check was performed by applying 50µl tritiated water (ca. 18.5 kBq) to the surface of the skin membranes. Portions of the receptor fluid were collected after 0.5, 1 and 2 hours. After 2 hours the skin was washed with deionised water to remove all remaining radioactivity and the preparations left in a water bath (32 ±2°C) overnight. The receptor fluid aliquots were then analysed for the amount of tritiated water which had penetrated in 2 hours. Skin samples with a penetration rate (Kp) of >$10x10^{-4}$ cm/h, were considered to have an

Section A6.2 (02) **Percutaneous absorption**

Annex Point IIA6.2 **In-vitro dermal absorption in human skin**

		altered membrane integrity to tritiated water, but not necessarily to the test substance. As the results of the membrane integrity check were not known at the start of the study, all physically undamaged cells received the appropriate test substance treatment. Inclusion criteria were applied at the end of the study. Membranes were rejected and omitted from subsequent calculations where visual inspection showed physical damage, permeability constants for tritiated water were above 3 µL ^3H_2O cm^2/h, recovery of radioactivity was outside the range 90-110% or where the absorption profile for the test substance was not seen to be time dependent.
3.3.2	Concentration of test substance	Two dose rates were used in the study. A high dose level of 100 g/L cypermethrin (1.0 mg/cm^2) was chosen to represent the undiluted EC formulation and reflects the potential dermal exposure during commercial mixing and loading. A low dose of 25mg/L cypermethrin (0.00025 mg/cm^2) was used to represent the spray dilution when used during agrochemical spraying operations. The mean doses applied were 0.997 mg/cm^2 for the concentrate and 0.00025 mg/cm^2 for the spray dilution.
3.3.3	Specific activity of test substance	See point 3.1.2.2
3.3.4	Volume applied	10 µl/cm^2 (applied as 17.7 µl using a positive displacement pipette)
3.3.5	Size of test site	1.77 cm^2
3.3.6	Exposure period	24 hours
3.3.7	Sampling time	All the receptor fluid was emptied from the Franz cell and replaced with fresh receptor fluid pre dose, then at 1, 2, 4, 6, 8, 10 and 24 h post dose. Fractions were stored at <-10°C prior to determination of radioactivity. At 8 h post application, residual formulation was washed from the surface of the skin by flushing the membrane with a solution of Liquid Ivory™ soap (*ca* 10% w/v) containing no organic solvent and rinsed with deionised water. Ethanol was added to the washings and these were retained for analysis. The washing procedure could not be rigorous as the skin membranes are friable and robust washing could damage the skin.
3.3.8	Samples	At 24 hours post-application (after the last receptor fluid sampling) the donor cell was removed and the surface of the skin was tape stripped 5 times by application of 3M scotch tape 810. The tape stripping removed the stratum corneum from the upper layers of the epidermis. The tape strips were retained for analysis. The Franz cells were dismantled and placed into a container where residual radioactivity was extracted with ethanol:water (50:50 v/v). The apparatus was removed from the containers and the washings retained.

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Following initial analysis, terminal receptor fluids, surface washings, solubilised membranes and diffusion cell washings were stored at <- 10°C.

3.3.9 Analysis of Radioactivity

Receptor fluid diffusion cell washings were added directly to liquid scintillant prior to LSC. Ethanol was added to the surface washings and portions added to liquid scintillant prior to LSC.

The skin membranes were removed from the cells and solubilised in Soluene 350. After an appropriate period liquid scintillant was added and the samples allowed to dark adapt prior to LSC.

The tape from the tape stripping was solubilised in Soluene 350 followed by the addition of acetonitrile. After an appropriate period liquid scintillant was added and the samples allowed to dark adapt prior to LSC

Radioactivity was measured for 5 min or for 2 sigma % using Packard Tri-Carb liquid scintillation counters (Canberra Packard) with the facilities for computing quench-corrected disintegrations per minute (dpm).

4 RESULTS AND DISCUSSION

4.1 Recovery of labelled compound

Results are expressed as ng equivalents of [^{14}C]-Cypermethrin cis:trans 40:60 absorbed per cm^2 of skin (ng equivalents/cm^2) and as a percentage of the applied dose. Calculations are based on the actual dose applied to each cell. For the purposes of this study, the absorbed dose is defined as the cumulative amount of radioactivity measured in the receptor fluid throughout the study (i.e. amount penetrated).

Concentrate (100g/L)

Following a 1 mg/cm^2 application of [^{14}C]-Cypermethrin cis:trans 40:60 to the human skin membrane, recovery of radioactivity was essentially quantitative. There was a mean lag phase of ca 2.1 hours prior to absorption of radioactivity. The mean maximum rate of absorption was 1553 ng/cm^2/h. The mean concentration/time curve showed that absorption slowed down 10 hours following dose application. The mean permeability coefficient (Kp) for [^{14}C]-Cypermethrin cis:trans 40:60 at this concentration was 1.6^{-5} cm/h.

Absorbed radioactivity, in the receptor fluid, accounted for 1.5% of the applied dose by the terminal timepoint, corresponding to a mean of 21990 ng equivalents/cm^2. The majority of the radioactivity was removed from the skin during the washing procedure at 8 hours following dose application (43.2%). The remainder of the radioactivity was recovered the tape strips (10.5%) or following solubilisation of the residual skin (25.5%). Residual radioactivity extracted from the diffusion chamber accounted for 17.4% of the applied radioactivity.

Spray Dilution (25 mg/L)

Following a 0.00025 mg/cm^2 application of [^{14}C]-Cypermethrin cis:trans 40:60 to human skin, recovery of radioactivity was essentially quantitative. There was a mean lag phase of ca 0.16 hours prior to absorption of radioactivity. The mean maximum rate of absorption was 3.328 ng/cm^2/h. The mean concentration/time curve showed that absorption was steady throughout the study and did not plateau. The

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mean permeability coefficient (Kp) for [^{14}C]-Cypermethrin cis:trans 40:60 at this concentration was 1.3^{-4} cm/h.

Absorbed radioactivity, in the receptor fluid, accounted for 13.1% of the applied dose by the terminal timepoint, corresponding to a mean of 42.7 ng equivalents/cm^2. The majority of the radioactivity was recovered following solubilisation of residual skin (55.6%). The remainder of the radioactivity was removed from the skin during the washing procedure at 8 hours following dose application (16.6%) or recovered from the tape strips (9.9%). Residual radioactivity extracted from the diffusion chamber accounted for 6.8% of the applied radioactivity.

See Tables A6.2_02_1 and A6.2_02_2.

4.2 Percutaneous absorption

Radioactivity was rapidly absorbed through human epidermal membranes, with levels detected in the receptor fluid at 1 hour after application. Absorption of radioactivity at study termination was minimal, accounting for 1.5% and 13.1% of the applied dose for the concentrate and spray dilution respectively (21990 and 42.7 ng equivalents/cm^2 for concentrate and spray dilution respectively). The amount absorbed increased ca 500 fold for a 4000 fold increase in exposure indicating that absorption of radioactivity was not proportional to increasing exposure and suggesting that the routes of absorption may have been saturated at the higher dose level. For the concentrate, there was a lag phase of ca 2.1 h. After the first four hours the rate of absorption increased until 10 hours then the rate of absorption slowed until termination. In contrast, for the spray dilution, there was a lag phase of ca 0.2 hours, then radioactivity was essentially absorbed proportionally with time.

The washing procedure, performed 8 hours following exposure, removed variable amounts of radioactivity. The washing procedure had no noticeable effect on the rate of absorption of radioactivity. For the concentrate, the majority of the radioactivity was removed during the washing procedure, whereas, for the spray dilution, a greater proportion remained associated with the skin. The cells in which the skin contained the greater amounts of radioactivity also had the greater amounts of radioactivity in the receptor fluid. This indicated that some of the radioactivity remaining on the skin following washing was available for absorption. The washing procedure could not be rigorous as the skin membranes are friable and robust washing could damage the skin. The complete removal of a compound is, therefore, doubtful and some of the compound found in the skin samples was probably due to [^{14}C]-Cypermethrin cis:trans 40:60 remaining on the surface of the membrane. Incomplete removal of highly lipophilic, low water soluble compounds by the washing procedure used in this study is not unexpected. It is probable that greater amounts of compound could have been removed by a more robust washing procedure and that the absorption of test substance could have been proportionally lower. For both groups ca 10% of the applied of radioactivity was associated with the stratum corneum which was removed by tape stripping.

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5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The in vitro dermal absorption of [^{14}C]-Cypermethrin cis:trans 40:60 was determined in human dermatomed membranes using a static cell system according to OECD guideline 428. Skin absorption was investigated at two application rates. The high dose rate, nominally 1.0 mg/cm^2, represented the undiluted EC formulation and therefore reflected a worst case operator exposure during mixing and loading. The low dose , nominally 0.00025 mg/cm^2, represented the diluted spray solution used in normal agricultural spraying operations.

The mean doses applied were 0.997 mg/cm^2 for the concentrate and 0.00025 mg/cm^2 for the spray dilution.

The dermatomed membranes were not occluded and the skin was exposed to the test substance for 8 h, after which time the skin was washed. Receptor fluid was collected up to 24 h post dose. At 24 h post dose the skin was tape stripped to remove the stratum corneum.

Radioactivity was determined in the receptor fluid, residual skin, skin washings, tape strips and diffusion cell washings to determine the overall mass balance of radioactivity.

5.2 Results and discussion

Recovery of radioactivity was essentially quantitative for both dose levels.

Absorption of radioactivity was rapid, with detectable levels in the receptor fluid at 1 h, but minimal, accounting for 1.5 and 13% of the applied dose for the concentrate (100 g/L) and spray dilution (25 mg/L) respectively.

The amount absorbed increased only ca 500 fold for a 4000 fold increase in exposure, suggesting that absorption was not proportional to increasing exposure indicating saturation of absorption at the higher dose level.

The washing procedure removed variable amounts of radioactivity. For the concentrate, the majority of applied radioactivity was removed but, for the spray dilution, the majority remained associated with the skin. The washing procedure had no noticeable effect on the rate of absorption of radioactivity.

Approximately 10% of the applied radioactivity was associated with the stratum corneum which was removed during the tape stripping process.

5.3 Conclusion

At the high dose level (nominal applied dose 1.0 mg/cm^2), 1.5% of the applied dose was absorbed through the skin. At the low dose level (nominal applied dose 0.00025 mg/cm^2), 13% of the applied dose was absorbed through the skin.

5.3.1 Reliability 1

5.3.2 Deficiencies No

Section A6.2 (02) Percutaneous absorption

Annex Point IIA6.2 **In-vitro dermal absorption in human skin**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	~~March, 2007~~ June, 2011.
Materials and Methods	The applicant's version is acceptable with the following amendments: Protocol deviations: The certificate of analysis for the non-radiolabelled test substance did not include details of physical appearance, known hazardous properties, purity, stability, and date of expire.
Results and discussion	The applicant's version is adopted with the following amendments: Exclusion of membranes: Two cells were rejected (1 low dose, 1 high dose) due to the poor procedural recovery of radioactivity following the application [^{14}C]-cypermethrin cis:trans 40:60. The residual amounts within the skin have to be included. It cannot be excluded that the amount present in the skin could not be potentially absorbed. Dermal absorption values including residual skin: Concentrate: **27%** Spray dilution: **68.6%** According to the general consensus at TM (2008) and Mota, the material found in the stratum corneum should also be included in the absorbed dose unless tape stripping data is available that allows to discount the top 25% of the stratum corneum. As there is no information for the tape strips individually, dermal absorption values including residual skin and all 5 tape strips: Concentrate: **37.5%** Spray dilution: **78.6%**
Conclusion	Dermal absorption values including residual skin: Concentrate: **27%** Spray dilution: **68.6%** According to the general consensus at TM (2008) and Mota, the material found in the stratum corneum should also be included in the absorbed dose unless tape stripping data is available that allows to discount the top 25% of the stratum corneum. As there is no information for the tape strips individually, dermal absorption values including residual skin and all 5 tape strips: Concentrate: **37.5%** Spray dilution: **78.6%** ~~The applicant's version is adopted.~~
Reliability	1
Acceptability	Acceptable.
Remarks	No results for relevant reference chemicals were made available: No appropriate reference substance was tested concurrently with the test substance. Nor was adequate historical data provided.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.*

Section A6.2 (02) Percutaneous absorption

Annex Point IIA6.2 **In-vitro dermal absorption in human skin**

	Discuss if deviating from view of rapporteur member state
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.2_02_1 Adsorption of radioactivity through human skin membranes

Time (h)	Mean cumulative absorption (ng/cm^2 skin)			
	Concentrate 1.0 mg/cm^2		Spray dilution 0.00025 mg/cm^2	
	Mean	SD	Mean	SD
1	470.3	229.4	2.819	1.081
2	1149	599.9	6.148	2.251
4	2595	1735	7.569	2.479
6	5702	4101	10.33	4.027
8	9276	6815	14.00	6.159
10	12775	8641	17.80	6.905
24	21990	13514	42.73	14.67
Maximum rate of penetration (ng/cm^2/h)	1553	1193	3.328	1.170
Permeability coefficient (cm/h)	1.6^{-5}	1.2^{-5}	1.3^{-4}	4.7^{-5}
Lag Time (hours)	2.141	0.431	0.161	0.032

Table A6.2_02_2 Recovery of radioactivity through human skin membranes

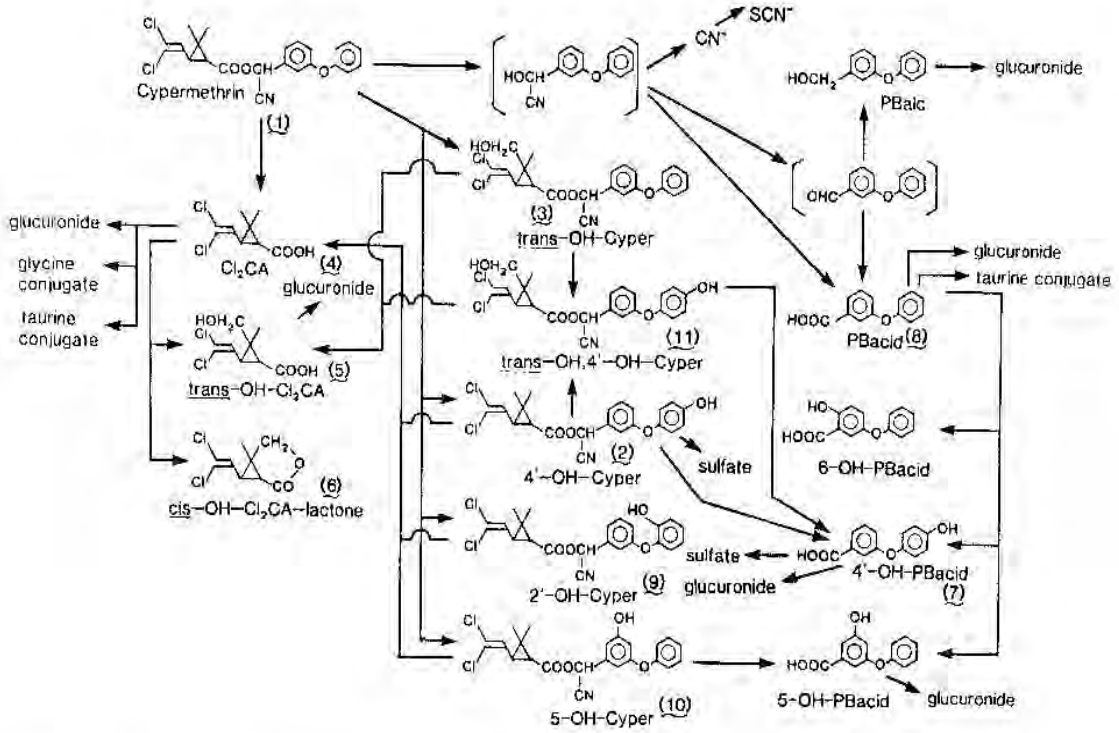
Sample	Mean recovery of radioactivity (% applied dose)			
	Concentrate 1.0 mg/cm^2		Spray dilution 0.00025 mg/cm^2	
	Mean	SD	Mean	SD
Receptor Fluid	1.491	0.885	13.13	4.672
Surface Wash	43.17	14.05	16.57	8.125
Skin	25.49	10.42	55.64	14.19
Cell Wash	17.42	8.348	6.810	2.687
Tape Strip	10.50	3.192	9.863	9.130
Total	98.06	3.410	98.23	4.942

Section A6.2 (03) **Annex Point IIA VI.6.2**	**Toxicokinetics/Metabolism** **Metabolism in mammals – nature of the metabolites**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [√]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Extensive metabolism studies with cypermethrin have been conducted in a number of species. These studies were peer reviewed by the International Programme on Chemical Safety (IPCS) and the conclusions of this expert group published in Environmental Health Criteria no.82 – Cypermethrin (WHO, 1989). A copy of this publication is included in Document IV (literature search). The conclusion of the IPCS was that the overall metabolism of cypermethrin was similar in each of the animals studied, based on existing in-vivo studies in rats, mice, dogs and cows. Differences related only to the rate of metabolism rather than the nature of the metabolites formed. The only major species differences related to the type of conjugation reactions which take place prior to elimination. Hydrolytic cleavage of the ester bond and elimination of the cis and trans cyclopropanecarboxylic acid and 3-phenoxybenzyl moieties in the free and conjugated form is known to be a major route of metabolism in mammals, including humans. The cyclopropane carboxylic acid moiety is mainly excreted as the glucuronide conjugate, with only limited hydroxylation of the methyl group. The cyanide moiety is metabolised to thiocyanate. The 3-phenoxybenzyl moiety is converted to 3-phenoxybenzoic acid and further conjugated and excreted as the glutamic acid conjugate in the cow, as a taurine conjugate in the mouse and as a glycine conjugate in the rat and dog. Phenoxybenzoic acid is further metabolised to a hydroxyl derivative (3-(4'-hydroxyphenoxy)benzoic acid) and conjugated with glucuronic acid or sulphate. The major route of excretion of metabolites is via the urine. In rats given a single toxic oral dose (200 mg/kg) of radiolabelled cypermethrin, the route of biotransformation of cypermethrin was equivalent to those described for sub-lethal doses of cypermethrin. The absorbed cis- and trans- isomers of cypermethrin were rapidly metabolised via cleavage of the ester bond to yield the cis- and trans-cyclopropane carboxylic acids which were then excreted mainly as glucuronide conjugates in the urine. The 3-phenoxybenzyl moiety was mainly converted to the 3-phenoxybenzoic acid, most likely via α-hydroxy-phenoxybenzonitrile, with sulphation being the main route of conjugation. There was no evidence accumulation of unknown metabolites following repeated exposure and no indication of sex or dose dependent changes (Rhodes et. al., 1984). The identification cypermethrin metabolites in mice has been carried out using radiolabelled forms of the separate cis- and trans- isomers. Radioactivity from the trans-isomer was mainly excreted in the urine and that from the cis-isomer in the faeces. As reported for other species, metabolism occurred mainly via ester cleavage and elimination of the cis- and trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane - carboxylic acid moieties as the glucuronide conjugates. The α-cyano-3-phenoxybenzyl alcohol released as a results of ester cleavage was mainly converted to 3-phenoxybenzoic acid which was partly eliminated unchanged, partly conjugated with amino acids (mainly taurine) and glucuronic acid and partly oxidised to 3-(4-hydroxyphenoxy) benzoic	

Section A6.2 (03) **Annex Point IIA VI.6.2**	**Toxicokinetics/Metabolism** **Metabolism in mammals – nature of the metabolites**
	acid which was then excreted as the sulphate conjugate (Hutson et al, 1981). In man, the ester cleavage of the cypermethrin molecule and subsequent elimination of the cyclopropyl acid moieties in the free and glucuronidated form is again the major route of metabolism. In a human dose excretion study, four male subjects were given a single oral dose of a 1:1 cis:trans mixture of Cypermethrin (0.25 mg to 1.5 mg). Urinary excretion of the free and conjugated 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid was rapid, occurring within the first 24 hours. Subjects excreted 78% of the trans- isomer dose and 49% of the –cis isomer dose in the form of metabolites (Eadsforth and Baldwin, 1983). A metabolic pathway for cypermethrin in mammals has been proposed by IPCS (see fig.1). Therefore it is considered that further work on the identification of metabolites in mammals is unlikely to produce significant new data which will affect the human health risk assessment. Results of a literature search specifically aimed at further information on the metabolites of cypermethrin is provided in Doc IV.A.6.2 along with copies of the published references outlined in this overview.
Undertaking of intended data submission [ ]	
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March, 2007.
Evaluation of applicant's justification	The applicant's justification is acceptable with the following amendments: Abstract out of the Environmental Health Criteria no.82 – Cypermethrin (WHO, 1989) Document. See at the bottom of the document.
Conclusion	The applicant's justification is acceptable. Extensive metabolism studies with cypermethrin have been conducted in a number of species. The open literature studies and unpublished study reports were peer reviewed by the International Programme on Chemical Safety (IPCS) and the conclusions of this expert group published in Environmental Health Criteria no.82 – Cypermethrin (WHO, 1989). A metabolic pathway for cypermethrin in mammals has been proposed by IPCS (see fig.1). Therefore it is considered that further work on the identification of metabolites in mammals is unlikely to produce significant new data which will affect the human health risk assessment. In conclusion, a reliable human health risk assessment can be made based on the available data and conclusions made in the Environmental Health Criteria no.82 – Cypermethrin document (WHO, 1989).
Remarks	

Section A6.2 (03) **Annex Point IIA VI.6.2**	**Toxicokinetics/Metabolism** **Metabolism in mammals – nature of the metabolites**
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Fig. 1.: Metabolic pathway for cypermethrin in mammals (WHO, 1982)



1. (RS)-α-cyano-3-phenoxybenzyl-(1RS)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate
2. 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid
3. 3-Phenoxybenzoic acid (3PBA)
4. (RS)-α-cyano-3-phenoxybenzyl-(1RS)-3-(2,2-dichlorovinyl)-2-hydroxymethyl-2-methylcyclopropane carboxylate
5. 3-(2,2-dichlorovinyl)-2-hydroxymethyl-2-methylcyclopropane carboxylate
6. N-(3-phenoxybenzoyl) taurine
7. N-(3-phenoxybenzoyl) glycine
8. N-(3-phenoxybenzoyl) glutamic acid
9. 3-(4-hydroxyphenoxy) benzoic acid
10. 4-(3-carboxyphenoxy)-phenyl sulphate
11. (RS)-α-cyano-3-(4-hydroxyphenoxy)-benzyl-(1RS)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate

Abstract out of the Environmental Health Criteria no.82 – Cypermethrin (WHO, 1989) Document.

6. KINETICS AND METABOLISM

6.1. Absorption, Excretion, and Distribution

6.1.1. Oral

6.1.1.1. Rat

(a) Cypermethrin mixture

Three rats of each sex were given a single oral dose of 0.5 mg (approximately 1.2 mg/kg body weight for males and 2.1 mg/kg body weight for females) of a cis/trans mixture of 14C-cyclopropyl-labelled cypermethrin. Three days after dosing, low concentrations of radioactivity were found for both sexes in the kidneys, muscle, brain, and blood. The level in the liver of male rats was 3 times higher than that in the liver of female rats (0.37 and 0.12 mg/kg tissue, respectively). The residues in the fat of the female rats were 2 - 3 times higher than those in the male rats (0.72 and 0.31 mg/kg tissue respectively). Concentrations in muscle, brain, and blood were < 0.05 mg/kg. The mean percentage recovery of the administered dose was more than 100% (Crawford, 1977; Crawford et al., 1981a).

Urinary excretion of the compound was rapid in both sexes; approximately 50 - 65% of the dose being excreted in 48 h. Elimination via the faeces was slower, the mean rate being approximately 30% of the dose in 3 days. The amount of radioactivity excreted via expired CO2, measured in a separate study using one rat of each sex, was up to 0.1% of the dose in 15 days.

Studies with 14C-cyclopropyl-labelled cypermethrin indicated that biliary excretion of the cyclopropyl moiety is a minor route of elimination (up to 2% in 4 h) (Crawford et al., 1981a).

The metabolism of cypermethrin in maize oil was studied in male and female Wistar rats following a single toxic oral dose of 200 mg/kg body weight of 2 radio-labelled forms (14C-benzyl and 14C-cyclopropyl) of the insecticide. Minimal amounts of 14CO2 were expired from both types of labelled cypermethrin: viz < 0.005 - 0.06% of dose. The elimination of radioactivity within 7 days was 29 - 33% (14C-benzyl label) and 41-56% (14C-cyclopropyl label) in the urine and 55 - 59% and 34 - 46%, respectively, in the faeces. The differences between the sexes were small (Rhodes et al., 1984).

The distribution and tissue retention of cypermethrin was studied in 5 male and 5 female Wistar rats receiving daily oral doses of 2 mg (14C-benzyl)-labelled cypermethrin/kg body weight for 28 days. Consistent with the lipophilic nature of cypermethrin, the highest mean tissue concentration was found in the fat (4.1 mg/kg in males and 5.1 mg/kg in females). Concentrations in the liver, kidneys, adrenals, gut, ovaries, and skin were of the order of 0.4 - 0.9 mg/kg tissue. Small amounts of radioactivity (0.04 - 0.07 mg/kg) were detected in the muscle, spleen, and bone.

Negligible concentrations (< 0.01 mg/kg) were detected in the

brain (Rhodes et al., 1984). In a further study, the tissues identified as containing the highest concentrations of 14C-benzyl-labelled cypermethrin (fat, liver, kidneys, skin, and ovaries) as well as whole blood and plasma were used to study the extent of accumulation and rate of elimination of cypermethrin. A total of 60 female rats were dosed orally with 14C-benzyl-labelled cypermethrin at 2 mg/kg body weight per day, for up to 70 consecutive days. Levels in all tissues reached a plateau after 56 days of dosing. The extent of accumulation, expressed as mg equivalents of cypermethrin per kg tissue, was: fat, 3.91; liver, 0.97; kidneys, 0.69; ovaries, 0.03; skin, 1.89; whole blood, 0.35; and plasma 0.64. Analysis of fat samples, 24 h after the final dose, revealed that higher levels of the cis-isomer of cypermethrin had been retained than of the trans-isomer. The rate of elimination of radioactivity from fat was biphasic in nature, with rapid elimination of trans-cypermethrin (half-life = 3.4 days) and slower elimination of the less-readily hydrolysed cis-cypermethrin (half-life = 18.9 days). Levels of 14C residues in the liver, kidneys, and blood reached control background levels within 29, 8, and 15 days, respectively, of the final dose. Apart from fat, the only other tissue that contained radioactivity was the skin; the rate of elimination of radioactivity from the skin was similar to that for fat. Accumulation in the sciatic nerve was also studied in rats dosed for 26 days. No appreciable bioaccumulation was found to occur (Jones, 1981; Rhodes et al., 1984).

Three Wistar rats of each sex, given a single oral dose of (14C-cyano)-cypermethrin (4.3 mg/kg body weight), eliminated 30 - 66% of the dose in the faeces over 3 days. Urinary excretion of 14CN-label was slow, accounting for 6 - 12% of the dose and elimination of expired 14CO2 accounted for only 1.2 - 1.5% of the dose. Tissue retention in major organs apart from fat, was higher than that in similar studies involving 14C-benzyl or 14C-cyclopropyl labelling, thus reflecting metabolism typical of the 14C-labelled cyanide moiety (Crawford et al., 1981a).

(b) Separate isomers

The fates of both cis- and trans-isomers have been studied separately. Groups of 3 - 6 Wistar rats of each sex were given single oral doses (approximately 2.5 mg/kg body weight) of either the cis-isomer or the trans-isomer, both 14C-labelled in the benzyl ring. Both isomers were rapidly eliminated. The greater part of the administered dose was excreted in the urine; 40% and 60% for males and females, respectively, of the cis-isomer and 70% and 80% of the trans-isomer within 48 h. Elimination of the cis-isomer in the faeces amounted to 26% and 48% for male and females, respectively; elimination of the trans-isomer was 24%. The results for the cis-isomer show a clear sex difference in the route of elimination. After 72 h, less than 5% of the administered dose of either isomer remained in the animal tissues with the exception of the intestines and skin. Fat and skin contained the highest concentrations (Crawford, 1976a,b; Crawford et al., 1981a). It has

been demonstrated (Crawford & Hutson, 1977a, Crawford et al., 1981a) that the residue derived from cis-cypermethrin is eliminated more slowly from fat than from other tissues. In one study, 8 female rats were given (14C-benzyl)- cis-cypermethrin at 2.5 mg/kg body weight orally, and elimination of radioactivity was measured in fat samples from 8 up to 42 days after dosing. The

radioactivity was calculated to have a half-life of 11.7 (3.4 - 16.7) days. Ninety to 100% of the radioactivity still remaining in the fat at 25 days was present as unchanged cypermethrin. The residues in the liver and kidneys were much lower than those in the fat but were eliminated at a similar rate (Crawford et al., 1981a).

6.1.1.2. Mouse

(a) Separate isomers

Elimination of radioactivity was measured in male Swiss-Webster mice, dosed once orally with cis- or trans-cypermethrin, 14C-labelled in either the benzyl (8 mg/kg body weight) or cyclopropyl (7 mg/kg body weight) moiety. The 14C-benzyl-dosed mice eliminated 22% and 34% of the administered dose of cis-isomer in the urine and faeces, respectively, in one day; values for the trans-isomer were 41% and 16%, respectively. The 14C-cyclopropyl-dosed mice eliminated 20% of the administered dose of cis-isomer in the urine and 50% in the faeces in one day; the values for the trans-isomer were 55% and 16%, respectively. Thus, radioactivity from the trans-isomer was mainly eliminated in the urine and that from the cis-isomer in the faeces. The 14C-benzyl-treated mice were killed 1, 3, or 8 days after dosing; the 14C-cyclopropyl-treated mice, 3 days after dosing. Residues of radioactivity from both labels, 3 days after dosing, were low in all tissues except for the fat. The sequence of the residues in different organs was fat > liver ~ kidneys > blood ~ muscle > brain. Residues fell rapidly during the 14C-benzyl study, with the exception of the residues derived from the cis-isomer in fat, which did not decrease during the study period (Hutson, 1978a; Hutson et al., 1981). However, in a further study, radioactivity was measured in fat samples from 10 male mice taken up to 42 days after a single oral dose of approximately 8.8 mg/kg body weight (14C-benzyl)- cis-cypermethrin. The residue was eliminated exponentially with a half-life of 13.1 (3.6 - 18.4) days. At 8 and 22 days after dosing, approximately 90% of the radioactivity present in two pooled fat samples was attributable to unchanged cis-cypermethrin (Crawford & Hutson, 1978; Crayford et al., 1980; Hutson et al., 1981).

6.1.1.3. Dog

(a) Cypermethrin mixture

Two male beagle dogs were given single oral doses of (14C-cyclopropyl)-cypermethrin at 2 mg/kg body weight (Crawford, 1979a). Elimination of labelled material was rapid in both dogs, though a variable distribution between urine and faeces was observed between the 2 dogs, i.e., 21 and 57% in urine and 78 and 48%, respectively, in faeces. In a further study, one dog was dosed orally with

(14C-benzyl)-cypermethrin at 2 mg/kg body weight (Crawford, 1979b). Over 4 days, 80% of the radioactivity was recovered in the faeces and 11% in the urine. Analysis of tissues, 4 days after dosing, revealed that the gall bladder (1.5 mg/kg tissue) and renal fat (0.3 mg/kg tissue) contained the highest levels of radioactivity expressed as cypermethrin. Negligible amounts were detected in the brain (0.006 mg/kg tissue) and sciatic nerve (0.09 mg/kg tissue). In the liver, adrenals, bone marrow, pituitary gland, and mesenteric fat, levels of cypermethrin of 0.1 - 0.2 mg/kg tissue were found.

(b) Separate isomers

Administration of (14C-benzyl)- cis-cypermethrin or (14C-benzyl)- trans-cypermethrin separately to groups of 2 male dogs as a single (2 mg/kg body weight) oral dose resulted in 83.4% of cis-isomer and 88% of trans-isomer being recovered in the urine plus faeces over 6 - 7 days (Crawford, 1979b). Quantitative differences existed between the amounts eliminated via the 2 routes. As already mentioned, a variable distribution was found. These data are consistent with the results of the study involving 14C-cyclopropyl-labelled cypermethrin (Crawford, 1979a), and the variation in amounts according to the route of elimination probably reflects the inter-group differences in rates of absorption of labelled material.

6.1.1.4. Cow

Three studies were carried out on lactating cows fed diets containing 0.2, 5, or 10 mg 14C-benzyl and/or 14C-cyclo-propyl-cypermethrin/kg feed, respectively, twice daily, for 7 or 21 days. The estimated daily intake was 2, 50, or 100 mg cypermethrin/cow. The radioactivity was rapidly eliminated following ingestion. Equilibrium between ingestion and elimination was reached after about 4 days. The amounts eliminated via the major routes were similar for both labels, i.e., approximately 50% in the urine, and approximately 40% in the faeces (mainly unchanged cypermethrin). Polar and acidic components were found in the urine. Up to 0.2% of the administered radioactivity was found in the milk, mainly in the cream phase (about 88%). Feeding 0.2, 5, or 10 mg/kg feed, the residues in the milk were 0.0006, 0.012, or 0.03 mg cypermethrin /litre, respectively. Radioactivity (expressed as mg cypermethrin /kg tissue) in the carcasses of the animals of the 3 groups at slaughter was not detectable in muscle and brain (< 0.001- < 0.04 mg/kg). Levels in other tissues were: blood < 0.04 - 0.07 mg/kg, liver 0.004 - 0.21 mg/kg, kidneys 0.003 - 0.11 mg/kg, and subcutaneous and renal fat 0.01 - 0.1 mg/kg (Croucher et al., 1985).

Swaine & Sapiets cf. FAO/WHO (1982b) dosed cows daily with 0.2, 5, or 50 mg cypermethrin (43% cis-isomers, 35% trans-isomers) per kg feed for up to 29 days. Residues in milk and tissues were comparable to those reported by Croucher et al. (1985).

6.1.1.5. Sheep

The elimination pattern in a single sheep, given one oral dose of a mixture consisting of unlabelled cypermethrin with 14C-benzyl- and 14C-cyclopropyl-labelled material (3.9 mg/kg body weight) in a gelatin capsule, showed that 41% of the administered dose was excreted in the urine and 20% was eliminated in faeces, within 48 h. Tissue residues, 2 days after treatment, were muscle, 0.04 mg/kg; and liver, kidneys, and renal fat approximately 0.4 mg/kg tissue (Crawford & Hutson, 1977b).

6.1.1.6. Chicken

14C-phenoxy-labelled cypermethrin (cis:trans, 55:45) was administered orally to laying hens, daily for 14 days, at a rate equivalent to 10 mg/kg diet (about 0.7 mg/kg body weight).

Radioactivity in the eggs reached a plateau, equivalent to about 0.05 mg cypermethrin/kg, after 8 days. Most of the radioactivity was found in the yolk (up to 0.19 mg/kg) and about half of it was identified as cypermethrin. The rest was closely associated with neutral lipids and phosphatidyl cholines. Residues in the carcasses, at slaughter, were low; values were between 0.01 and 0.02 mg/kg in muscle tissue, about 0.08 mg/kg in the subcutaneous and peritoneal fat, and 0.37 mg/kg in the liver. The composition of residues in the liver was not conclusively established. Apart from small amounts of unchanged cypermethrin, the radioactivity was also associated with highly polar material. However, it is evident that the hen has a very effective mechanism for the metabolism of cypermethrin (Hutson & Stoydin, 1987).

Comparable results were obtained from non-labelled studies with laying hens in which dietary levels of up to 40 mg cypermethrin/kg diet were fed for 28 days (Wallace et al., 1982).

6.1.1.7. Man

Male volunteers were each given a single oral dose of 0.25, 0.5, 1, or 1.5 mg cypermethrin in corn oil in a capsule. Urinary excretion of cypermethrin metabolites was rapid. The subjects excreted an average 78% of the dose of trans-isomer and 49% of the cis-isomer within 24 h. These values did not differ from the results in rats. The ester cleavage was a major route of metabolism of cypermethrin in man. As reported in other animal species, the trans-isomer was metabolized more readily than the cis-isomer. Concentrations of both isomers excreted in the urine between 2 and 5 days after dosing 0.5 or 1 mg cypermethrin were below the limit of detection of 0.01 mg/litre (Eadsforth & Baldwin, 1983).

Groups of 2 male subjects were given cypermethrin in daily oral doses of 0.25, 0.75, or 1.5 mg/man, by capsule, for 5 consecutive days. During the dosing period and the following 5 days, 24-h urine samples were collected daily and analysed for the concentration of the cyclopropane carboxylic acid metabolite. The results showed that the respective percent-ages of the cis- and

trans-isomers of cypermethrin, excreted in the 24-h period following each of the oral doses, were similar to the percentage excretion of these isomers measured in the single oral dose study. Therefore, no accumulation in the body occurred (van Sittert et al., 1985a).

6.1.2. Dermal

6.1.2.1. Cow

Two lactating cows were sprayed 3 times with 1.1 g cypermethrin/animal, with 2-week intervals between treatments. Milk samples were analysed during this period. Tissue samples were analysed approximately three weeks after the final spraying. The residues were: in whole milk, < 0.01 mg/litre; muscle, liver, and kidneys, < 0.01 mg/kg tissue and in fat samples, 0.02 mg/kg tissue or less (Baldwin et al., 1977).

Comparable results were obtained when 2 barns were sprayed with either 0.05% or 0.1% of cypermethrin prepared from a 10% a.i.

formulation. Cows were present during spraying. Milk was collected up to 4 weeks after spraying (0.05% application) or 4 days after spraying (0.1% application). Only the samples collected 4 days after the 0.05% treatment and 2 days after the 0.1% treatment contained detectable residues (0.005 mg/kg milk). No residues were found (< 0.002 mg/kg milk) in any of the other samples (Baldwin & Lad, 1978a).

Cows were dipped twice in approximately 170 mg cypermethrin /litre with a 10-week interval between treatments. The animals were sacrificed 4 or 14 days after the second dipping. Residues in muscle and liver did not exceed 0.01 mg/kg tissue. Fat samples contained detectable residues. The highest was 0.13 mg/kg in renal fat. The fat residue did not decline between 4 and 14 days after treatment (Baldwin, 1977a).

Cattle sprayed once with 0.1 and 0.2% a.i. showed the same level of residues (< 0.005 mg/kg tissue) in muscle, liver, and kidneys, and a level of < 0.01 mg/kg in fat samples, 1, 3, 8, and 15 days after treatment. In cattle treated twice, fat samples contained residues ranging from 0.01 to 0.05 mg/kg tissue (Bosio, 1979).

Many trials in which cows were sprayed with, or dipped in, cypermethrin solutions were carried out in Australia. The milk from cows sprayed with 0.1% cypermethrin did not contain any detectable residues. The highest residue (0.03 mg/kg) in butterfat was found one day after spraying. When the cows were dipped in a dipwash containing 75 mg cypermethrin/litre, residues in the milk determined 1, 3, and 7 days after dipping ranged from 0.01 to < 0.002 mg/litre. Omental fat contained the highest residue level (0.02 mg/kg) 3 and 4 days after dipping. Liver, kidneys, and muscle did not contain any detectable residues. A second dipping, 7 days after the first, did not cause any build-up of cypermethrin in the tissues of the cattle (FAO/WHO, 1982b).

Detectable residues of cypermethrin of up to 0.01 mg/kg butterfat were found in milk samples taken over 21 days from 5 of 10 cows wearing cypermethrin-integrated ear tags (Braun et al., 1985).

Taylor et al. (1985) found cypermethrin in the hair of cattle, in concentrations of up to 2.8 mg/kg, after application of impregnated ear tags.

6.1.2.2. Sheep

Two sheep were each treated dermally with a mixture consisting of unlabelled cypermethrin mixed with 14C-benzyl-and 14C-cyclopropyl-labelled material at 22 mg/kg body weight. The cypermethrin was slowly absorbed. Less than 0.5% of the dose was excreted in the urine within 24 h and only 2% over a 6-day period. Faecal elimination was also slow, 0.5% of the dose being eliminated in 6 days. Approximately 30% of the dose was recovered from the application area. Tissue residues, 6 days after treatment, were: muscle, 0.04; renal fat, 0.3; and liver and kidneys 0.12 mg/kg tissue (Crawford & Hutson, 1977b).

6.1.2.3. Man

A male subject was given a single dermal application of a ULV formulation of cypermethrin (50 mg cypermethrin in hexylene glycol/Shellsol AB) on the underside of the forearm. The majority of this application (35 mg) was removed from the skin after 4 h. Urine was monitored for residues of the acid metabolite [3-(2,2-dichlorovinyl)-2,2-dimethylcyclo-propane-carboxylic acid] and its glucuronide, for a 96-h period after dosing. The metabolites were not detected over this period (Coveney & Eadsforth, 1982).

In a study by van Sittert et al. (1985b), 2 male volunteers were given a single dermal application of a ULV formulation, 25 mg cypermethrin in hexylene glycol/Shellsol A, on the underside of the forearm. An average of 53% of the original amount of cypermethrin applied was removed from the skin, 4 h after application. Approximately 0.1% was excreted as the urinary metabolite, cyclopropane carboxylic acid, during a 72-h period. Measurements were made using gas liquid chromatography - mass spectrometry, a method with a higher sensitivity and selectivity than gas liquid chromatography - electron capture detection, which was used in the previous study.

6.2. Metabolic Transformation

6.2.1. In vitro studies

In vitro studies on mouse liver homogenates have shown that ester cleavage is more extensive for the trans-isomer than for the cis-isomer. One mg of each of (1RS,trans)- and (1RS, cis)-cypermethrin was incubated with 2.2 ml of approximately 10% mouse microsome substrate at 37 °C for 30 min, under the following conditions: (a) tetraethyl pyrophosphate (TEPP)-treated microsomes

(neither esterase nor oxidase activity); (b) normal microsomes (esterase activity); (c) TEPP-treated microsomes plus NADPH (oxidase activity); and (d) normal microsomes plus NADPH (esterase plus oxidase activity). Each esterase preparation hydrolysed about twice as much trans-cypermethrin as cis-cypermethrin. In contrast, cis-cypermethrin was metabolized more rapidly in an oxidation system than trans-cypermethrin. The major site of ring hydroxylation was the 4' position and the secondary site was the 5 position. The trans-methyl group was an important site of hydroxylation in the ester metabolites and cis-methyl oxidation was predominant in the ester-cleaved acid metabolites. The hydroxymethyl derivatives were further oxidized to the corresponding aldehydes and carboxylic acids.

3-Phenoxybenzaldehyde-cyanohydrin was detected as a minor metabolite. The preferred sites of hydroxylation were: with trans-cypermethrin, cis-methyl > 4' position > trans-methyl > 5 position; with cis-cypermethrin, trans > cis > 4' position > 5 position (Shone & Casida, 1978; Shone et al., 1979). With cis-cypermethrin, at least, cleavage of cypermethrin to cyanohydrin may result from both hydrolytic and oxidative mechanisms, since large amounts of the cleavage products were also evident in the oxidase system, which lacks esterase activity (Shono & Casida, 1978; Shono et al., 1979). However, at approximately 35-times higher substrate levels, the hydrolysis rate of cypermethrin isomers was depressed (Söderlund & Casida, 1977).

In studies on the metabolism of 14C-cypermethrin by rat liver

microsomes, the overall rates of metabolism of cis- and trans-cypermethrin were similar, though their metabolic routes differed. The cis-isomer was metabolized almost exclusively by an NADPH-dependent oxidative pathway to 4'hydroxy- cis-cypermethrin with subsequent oxidative ester cleavage. The predominant route for the metabolism of the trans-isomer was hydrolysis to the trans-acid by microsomal carboxylesterase (Crawford, 1979c). The in vitro esteratic capacity was determined in rat, rabbit, and human liver microsomes using p-nitrophenyl acetate and cypermethrin as substrate. The relative ability to hydrolyse cypermethrin was rabbit > man > rat. Rabbit and rat microsomes metabolized the trans-isomer 6 times faster than the cis-isomer. Human microsomes showed a similar capacity for metabolizing both cis- and trans-isomers (Croucher et al., 1982a,b).

6.2.2. In vivo studies

The identification of the metabolites of cypermethrin has been studied in mice (Hutson, 1978b, Casida et al., 1979; Hutson et al., 1981), rats (Crawford & Hutson, 1977a; Casida et al., 1979; Hutson, 1979a,b; Crawford et al., 1981b; Rhodes et al., 1984), dogs (Crawford, 1979d,e), and cows (Swaine & Sapiets, 1980a,b; Croucher et al., 1985).

Overall, metabolism in these species is similar. Differences that occur are related to the rate of metabolite formation rather than to the nature of the metabolites formed. The only major differences between species relate to conjugation reactions.

Cypermethrin (both isomers) is metabolized via cleavage of the ester bond. The cyclopropane carboxylic acid moiety is mainly excreted as the glucuronide conjugate; hydroxylation of the methyl group occurs only to a limited extent (Crawford, 1979e; Rhodes et al., 1984). The 3-phenoxy-benzyl product of the ester hydrolysis is converted to PBA. The cyanide moiety is metabolized to thiocyanate (Hutson, 1979b). The PBA moiety is mainly excreted as a glutamic acid conjugate in the cow (Croucher et al., 1985), as a taurine conjugate (N-(3-phenoxy-benzoyl)taurine) in 2 strains of mouse (Hutson & Casida, 1978; Hutson, 1978b, 1979a; Hutson et al., 1981), and as a glycine conjugate in the rat and dog (Crawford & Hutson, 1977a; Crawford, 1979d) and in the sheep, cat, and gerbil (Huckle et al., 1981a). PBA is further metabolized (rat > mouse > dog) via the 4'-hydroxylation to 3-(4'-hydroxyphenoxy) benzoic acid and its sulfate conjugate (Crawford & Hutson, 1977a; Hutson, 1978b; Crawford, 1979d). Glucuronic acid conjugates of PBA and its 4'hydroxy derivative are the major urinary metabolites in the marmoset, rabbit, guinea-pig, and hamster. The rat was unique among the animal species tested in utilizing sulfuric acid for the conjugation of the 4'-hydroxy derivative (Huckle et al., 1981a). The major route of excretion for cypermethrin metabolites is via the urine; unchanged cypermethrin accounted for the majority of radioactivity found in faeces in radiolabel studies. The amount of cyclopropyl-radioactivity eliminated in the bile (1%) suggests that the biliary-intestinal-faecal route is of minor importance for this moiety (Crawford & Hutson, 1977a; Crawford, 1979e; Rhodes et al., 1984). Biliary excretion of PBA occurred as glucuronide and that of 4'-OH-PBA as ether and ester glucuronic acid conjugates. Very little was eliminated in the faeces, indicating that the biliary glucuronides decompose and/or are enzymatically cleaved in the gastro-intestinal tract to the respective benzoic acids. The

latter are subsequently reabsorbed and undergo further metabolism, principally to the sulfate ester, which is excreted in the urine (Huckle et al., 1981b). As already noted, the major urinary metabolite of cypermethrin in cows is N-(3-phenoxy-benzoyl)glutamic acid. This metabolite is also found in the organs and tissues with only a small quantity of unchanged cypermethrin. The residues in body fat consist mainly of cypermethrin. An unidentified polar metabolite, present in the liver and kidneys, is suspected of being a conjugate of 3-(4'-hydroxyphenoxy)benzoic acid. The small portion of radioactivity appearing in milk was associated with lipid components and consisted mainly of unchanged cypermethrin (Croucher et al., 1985). The metabolic pathway of cypermethrin is shown in Fig. 3.

As in other mammals, ester cleavage and elimination of the cyclopropyl acid moieties in the free and glucuronidated form is a major route of metabolism of cypermethrin in man (Eadsforth & Baldwin, 1983).

6.2.3. Metabolism of the glucoside conjugate of 3-phenoxy-benzoic acid

Studies have been carried out on rats on the metabolism of the glucoside conjugate of 3-phenoxybenzoic acid, which occurs occasionally as a metabolite in plants (Crayford, 1978). The

results indicated that the rat hydrolyses the glucoside and then metabolizes the 3-phenoxybenzoic acid in virtually the same way as it would metabolize PBA liberated during the metabolism of cypermethrin.

The same conclusion was also reached by Mikami et al. (1985). During this study involving the metabolism of the glucoside conjugate of PBA, it was noticed that the skin and carcasses contained high residues (4 - 7% of the administered dose) of radioactivity. To characterize the metabolites of PBA in the skin and carcasses, rats were given (14C-benzyl) 3-PBA in a single oral dose (0.8 mg/kg body weight) or a higher dose (totalling approximately 750 mg/kg body weight) for 7 consecutive days. Two components were identified in the skin: unchanged PBA and a mixture of 3-phenoxybenzoyl-dipalmitins. The components were present in the skin of the high-dose animals in the approximate ratio of 3:7 and in the carcasses at 9:1 (Crayford & Hutson, 1979, 1980).

References available 'Open literature' publications:

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Söderlund, D.M., Casida, J.E. (1977). Effects of pyrethriod structure on rates of hydrolysis and oxidation by mouse liver microsomal enzymes. Pest; Biochem. Physiol., 7: 391-401.

Taylor, S.M., Elliott, C.T., Blanchflower, J. (1985). Cypermethrin concentrations in hair of cattle after application of impregnated ear tags. Vet. Rec., 116 (23): 620.

Unpublished research reports mentioned in the Environmental Health Criteria no.82 – Cypermethrin (WHO, 1989) Document were not made available to the BE CA.

Section A 6.2 (04) Toxicokinetics/Metabolism

Annex Point IIA VI.6.2 **Metabolism investigation in human volunteers**

Official use only

1 REFERENCE

1.1	**Reference**	Woollen, B.H., Marsh, J.R., Laird, W.J.D. and Lesser, J.E. (1992); The metabolism of Cypermethrin in man: differences in urinary metabolite profiles following oral and dermal administration. Xenobiotica, 1992, Vol 22, No 8, 983-991 (published).
1.2	**Data protection**	No
1.2.1	Data owner	Public domain information.
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No. Two human volunteer studies were conducted based on protocols that were in compliance with the Declaration of Helsinki, Tokyo and Venice Amendments
2.2	**GLP**	No
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	Cypermethrin technical. *cis:trans* ratio 1:1 for oral administration and 56:44 for dermal administration
3.1.1	Lot/Batch numbers	No details provided
3.1.2	Specification	For the oral study Cypermethrin obtained from ICI Agrochemicals was provided as four diastereoisomeric pairs with purity values of between 98.9 and 99.4%. These were dissolved in absolute ethanol to a concentration of 38 mg/mL for oral administration. Technical Cypermethrin was obtained for the dermal study as 91.5% (cis:trans ratio of 56:44), mixed with surfactants and wetting agents and dispersed in soya bean oil for administration
3.1.2.1	Description	No details provided
3.1.2.2	Purity	98.9-99.4 % w/w (Cypermethrin, *cis:trans* ratio 1:1) for oral study. 91.5% w/w (Cypermethrin technical, *cis:trans* ratio 56:44) for dermal study
3.1.2.3	Stability	No details provided
3.1.2.4	Radiolabelling	Not applicable
3.2	**Test Animals**	
3.2.1	Species	Human volunteers
3.2.2	Strain	Not applicable
3.2.3	Source	Not applicable

Section A 6.2 (04) Toxicokinetics/Metabolism

Annex Point IIA VI.6.2 **Metabolism investigation in human volunteers**

3.2.4	Sex	Males
3.2.5	Age/weight at study initiation	61-80 kg; 20-32 years old
3.2.6	Number of animals per group	6 male volunteers for oral study, 4 of these were also included in the dermal study, (which also consisted of a total of 6 volunteers).
3.2.7	Control animals	No
3.3	**Administration/ Exposure**	Oral and dermal
3.3.1	Type	Gavage and topical exposure
3.3.2	Concentration of test substance	Dose selection took into account the proposed ADI of 0.05 mg/kg bw/day for Cypermethrin as indicated by FAO/WHO 1982 and the fact that low concentrations of the expected metabolites (DCVA, 3PBA and 4OH3PBA) can be detected in urine. Dose concentrations were therefore selected to facilitate detection of the major identified metabolites of Cypermethrin and to establish the relationship between the urinary metabolites following oral or dermal administration. For oral administration the *cis-trans* diastereoisomeric pairs were prepared at 38 mg/mL. For dermal administration, technical Cypermethrin was dispersed in surfactants, wetting agents and soya bean oil at 26 mg/mL.
3.3.3	Preparation of test substance	For the oral study the dose was prepared from four diastereoisomeric pairs with a purity in the range of 98.9 to 99.4%. The *cis:trans* ratio was 1:1. Cypermethrin was dissolved in ethanol to give a final concentration of 38 mg/mL. The single oral dose was administered by adding 86µL of the dose solution to a sugar cube, allowing to air-dry for 20 minutes to allow ethanol to evaporate. The treated cube was swallowed, followed by 200 mL of water. The volunteers were allowed a light breakfast an hour after dosing (having been fasted overnight prior to treatment) and were then monitored for 24 hours. For the dermal study, technical Cypermethrin, purity 91.4% , *cis:trans* ratio 56:44, was mixed with wetting agents and surfactants and dispersed in soya oil to a concentration of 26 mg/mL. The dermal application site, (an area of 800 cm^2) on the dorsum of each volunteer was divided into 16 rectangular areas each of 50 cm^2. 75µL were applied to each of these 16 areas using a micropipette, such that a total volume of 1.2 mL was applied over the entire dorsal site. The area remained unoccluded for 8 h and was then washed with 1 mL of 3% aq. Teepol. The volunteers then wore a T-shirt until taking a shower 24 hours after dosing when the site was washed with soap and water.
3.3.4	Dose volume	For oral administration, 86 µL of dose formulation was added to a sugar cube, which the volunteer swallowed, followed by 200 ml of water. For the dermal application 1.2 mL was applied over 16 rectangular patches at 75µL/50 cm^2.
3.3.5	Dose administration	Single doses were administered by both routes.
3.3.6	Sampling time	Collection of urine samples – for both sets of volunteers urine was collected over the periods 0-4, 4-8, 8-12 h post treatment and then over 12h intervals up to 120 hours after dosing. Total volume, creatinine concentration and pH were analysed in the samples together with linear regression analysis of half-life – assessed on excretion rate versus mid-

Section A 6.2 (04) **Toxicokinetics/Metabolism**

Annex Point IIA VI.6.2 **Metabolism investigation in human volunteers**

point time from 18 h post treatment to the point at which metabolites fell below the limit of detection.

3.3.7 Tissue analysis

Urinary metabolites of cypermethrin (DCVA, 3PBA, 4OH4PBA and 2OH3PBA) were analysed to determine any differences following exposure via two different routes together with internal standards 4PBA and 4OH4PBA. The rat metabolite, 2OH3PBA, was not detected in human samples. The limit of detection was typically 0.5µg/L for all four metabolites and the precision is cited in the publication to be typically 5% relative standard deviation.

3.3.8 Treatment of samples

Hexane extracts of the swabs and T-shirt covers used by the volunteers were assessed using HPLC – using a Partisil PS1395 column with 1% acetonitrile in hexane mobile phase and u.v. detection at 230 nm. Recovery of radiolabelled Cypermethrin was assessed quantitatively.

Urine samples were treated to assess recovery of metabolites. Urine samples were spiked with 4OH4PBA and 4PBA {4-(4-hydroxyphenoxy) benzoic acid and 4-phenoxybenzoic acid} in methanol, acidified, heated and, following cooling, were roller mixed with diethyl ether. After centrifugation the ether layer was transferred to a Reacti-vial, washed with diethyl ether and washings added to the extract which was then dried in a nitrogen stream.

The carboxylic groups were then esterified by addition of pentafluoropropionic anhydride and 1H, 1H-pentafluoropropanol. Trifluoroacetic acid was added to completely acrylate the phenolic groups. The samples were then prepared for quantitative GLC-MS spectrometric analysis. Helium carrier was used in a fused silica capillary column, the detector was a VG TRIO 1 quadropole mass spectrometer. Selected ion recording was used to identify DCVA, 3PBA, 4PBA, 4OH4PBA and 4OH3PBA. 4PBA and 4OH4PBA were used as internal standards.

The Cypermethrin metabolite, 2OH3PBA, found in rats, was also assayed but was not detected in human urine samples following oral or dermal administration.

4 RESULTS AND DISCUSSION

4.1 Excretion Balance study

Not investigated

4.2 Radioactivity in Urine samples

Oral Dose (3.3 mg Cypermethrin)

Absorption of Cypermethrin was rapid and peak excretion rates were seen in the first 4 hours after dosing for the hydrolysis products – *cis* and *trans* DCVA. For the oxidised metabolites 3PBA and 4OH3PBA, the peak rates were seen between 4 and 24 hours after dosing. On average, 93% of recovered metabolites were excreted within the first 72 hours after dosing. For the majority of individual volunteers, some or all of the metabolites were still detectable in urine at five days after dosing, although the concentrations were approaching the limit of detection. Excretion rates for all four metabolites were similar when individual volunteer data were assessed. The elimination half life for total metabolites was 16.5 hours.

The *trans/cis* DCVA ratio was 2:1 on average and the total amounts of recovered DCVA and total phenoxybenzoic acid in urine were similar. The absorbed proportion of administered dose was estimated based on the total recovery of trans DCVA – mean 36% (range of estimate 27-

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57%).

Dermal Dose (31 mg Cypermethrin)

41% of the dermal dose (range 36-48%) was recovered in the detergent skin wash after the completion of the 8 hour exposure period. Extracts from the T-shirt cover used overnight post exposure produced a further 24% of applied dose. On average in this study at least 65% of the applied dose was not absorbed. Cypermethrin metabolites were detectable in the majority of urine samples over the first four hours of exposure but peak excretion rates occurred between 12 and 36 hours post dosing. No metabolites were detected beyond the 96 h sampling point (except for trace amounts of 4OH3PBA in two individuals). The elimination half life for total metabolites was 13 h (range 8-22h, standard deviation ±5.1 h). Four individual volunteers took part in both the oral and dermal phases of the assay, these individuals had similar elimination half lives for both exposure routes.

The average *trans:cis* DCVA ratio was 1:1.2. The amounts of cyclopropane acid metabolites in urine samples following dermal application were circa four times lower than the metabolites derived from the phenoxybenzyl moiety.

The estimate of Cypermethrin dermal absorption, based on *cis* or *trans* DCVA metabolite presence, was 0.3%, this was much lower than the same estimate based on 3PBA and 4OH3PBA – mean of 1.2% dermal absorption estimated.

4.3 Radioactivity in tissue samples

Not applicable – only urine samples assayed

4.4 QWBA – Tissue distribution study

Not applicable – only urine samples assayed.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Cypermethrin, a pyrethroid insecticide, was administered by oral gavage or by topical application to six male human volunteers as a single dose of 3.3 mg orally or 31 mg/800cm^2 by the dermal route. A soya oil based formulation was used for both administration routes.

For the oral study the dose was prepared from four diastereoisomeric pairs with a purity in the range of 98.9 to 99.4%. The *cis:trans* ratio was 1:1. Cypermethrin was dissolved in ethanol to give a final concentration of 38 mg/mL. The single oral dose was administered by adding 86µL of the dose solution to a sugar cube, allowing to air-dry for 20 minutes to allow ethanol to evaporate. The treated cube was swallowed, followed by 200 mL of water. The volunteers were allowed a light breakfast an hour after dosing (having been fasted overnight prior to treatment) and were then monitored for 24 hours.

For the dermal study, technical Cypermethrin, purity 91.4% , *cis:trans* ratio 56:44, was mixed with wetting agents and surfactants and dispersed in soya oil to a concentration of 26 mg/mL. The dermal application site, (an area of 800 cm^2) on the dorsum of each volunteer was divided into