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Toxicokinetics/Metabolism

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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.

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

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.

5.2 Results and discussion

No clinical effects of treatment were apparent during the oral or dermal studies or during the medical examinations conducted after completion of the study phase.

Excretion of urinary metabolites following a single oral administration of 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 27-57%).

Recovery of Cypermethrin and excretion of urinary metabolites following a single dermal application of 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

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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. 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 & Baldwin, 1983).

A metabolic pathway for Cypermethrin in mammals has been proposed by IPCS (see fig.1).

5.3 Conclusion

In the oral study reported in this paper, circa twice the amount of *trans*-DCVA as *cis*-DCVA was excreted in urine. The author assumes no *cis* to *trans* conversion has occurred in this process and that the *trans* DCVA excretion therefore represents the maximum estimate of absorbed Cypermethrin. Previous studies estimated urinary excretion to be 78% of administered *trans*-DCVA over a 24 h period, the estimate in this study is lower, at 27-57% with a mean value of 36%. The previous study administered the test material in solution in corn oil, the higher absorption may be related to the very lipophilic nature of Cypermethrin – slow dissolution in the gut may lead to lower GI tract absorption when no vehicle is used. The current results are supported by a similar study from the Wellcome Research Laboratories in which permethrin was administered without dissolution in a vehicle and 35% of total DCVA was recovered in urine over the following 72 hours. In other reported findings, orally administered Cypermethrin, given undiluted to dogs resulted in 80% excretion of radioactivity in faeces and only 11% recovered from urine.

The ratio of urinary metabolites following oral administration showed on average 1:0.8 for DCVA / (3PBA + 4OH3PBA) indicating more of the DCVA part of the molecule was accounted for in comparison with the phenoxybenzyl moiety and may indicate that a greater proportion of the PB moiety is metabolised to unmeasured metabolites.

In the dermal study the *trans:cis* ratio for DCVA was between 0.85 and 1.2 with an average of 1.0. In previous studies the DCVA ratio was not reported for dermal administration but it was confirmed that the two DCVA isomeric forms were present in approximately equal amounts in

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

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urine following dermal application – similar to current findings.

The cyclopropane acid metabolites were circa four times lower than those derived from the phenoxybenzyl moiety, indicating the cyclopropane acid may not be effectively absorbed or is converted to other metabolites that were not measured in this assay.

Cypermethrin absorption was estimated, from the recovered cyclopropane acids, to be 0.3% - similar to the 0.1% value obtained in a previous dermal study, (despite various differences in study design – e.g. vehicle, application site and treated area). However it is considered that the 1.2% absorption figure, based on phenoxybenzoic acid metabolites is a more reliable estimate of Cypermethrin dermal absorption. This value also accords well with the mean of 1.2% for radioactivity recovered in urine over day 0-5 following dermal application of permethrin in human volunteers.

It is concluded that the differences identified in this publication for recovery and isomeric ratio for cyclopropane acids after oral or dermal administration cannot be readily explained. In *in vitro* studies *cis*-Cypermethrin is more resistant to liver esterase hydrolysis than *trans*-Cypermethrin. *cis*-Cypermethrin is stored in adipose tissue in the rat and has a half life of 11.7 days – it is possible that significant metabolism occurs in the skin before Cypermethrin reaches the systemic circulation. It is possible that significant metabolism occurs in the skin leading to formation of metabolites that may not be formed following oral administration or the cyclopropane acids may be cleaved during transdermal absorption and retained in the skin while the phenoxybenzyl moiety is systemically absorbed.

Earlier animal studies showed large interspecies differences in the relative proportion of phenoxybenzoic acid metabolites. The formation of the 4OH3PBA conjugates increase in the order rat>mouse>dog and from this study these conjugates are less in man than in the rat.

Two important differences in urinary metabolite excretion are highlighted when Cypermethrin is administered orally and dermally. The *trans:cis* DCVA differs – oral 2:1 but dermal 1:1.2; the ratio of total DCVA/phenoxybenzoic acid metabolites differs – oral 1:0.8 and dermal 1:4.

Using total DCVA for biological monitoring is likely to underestimate Cypermethrin absorption following dermal application.

Using total phenoxybenzoic acid metabolites is the best estimate of total Cypermethrin absorption by all routes.

5.3.1	Reliability	2
5.3.2	Deficiencies	None

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Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

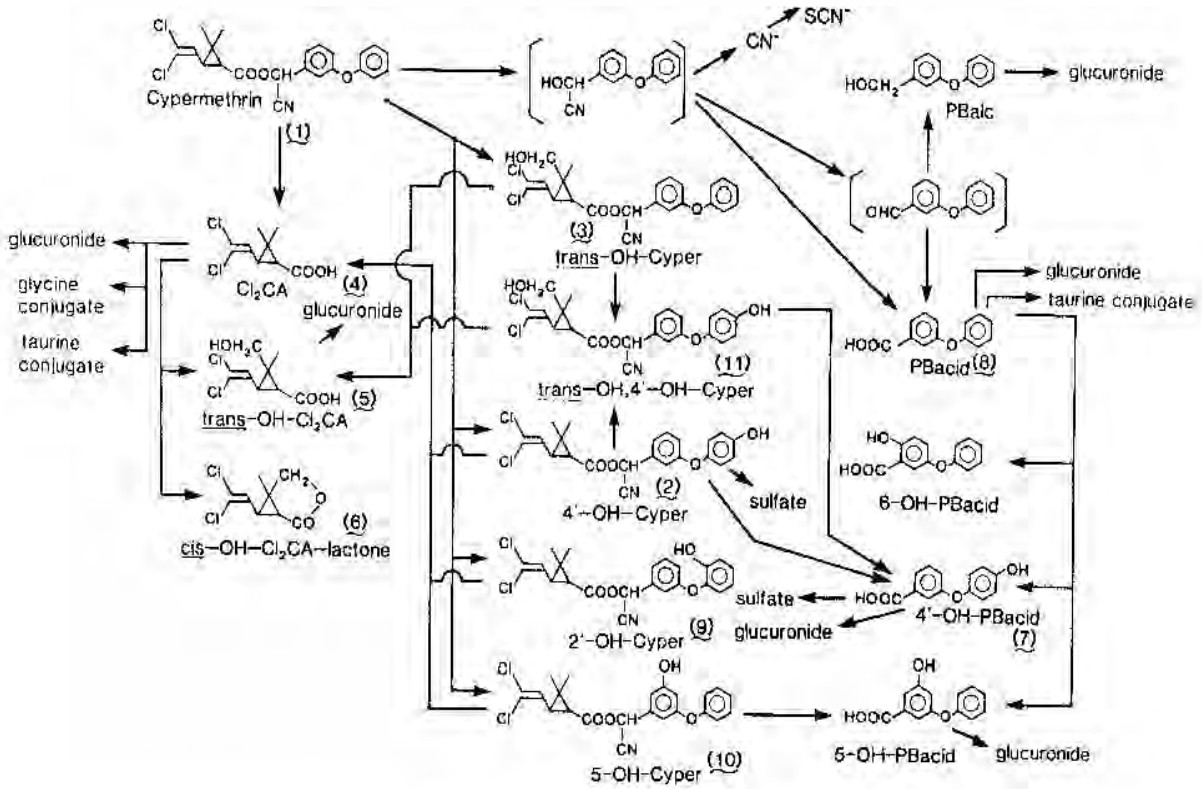
Date	January, 2011.
Materials and Methods	The applicant's version is acceptable.
Results and discussion	The applicant's version is adopted.
Conclusion	The applicant's version is adopted.
Reliability	2
Acceptability	acceptable
Remarks	-

COMMENTS FROM ...

Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

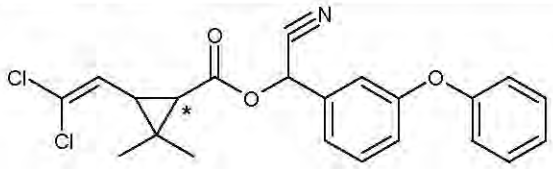
Extensive metabolism studies with Cypermethrin have been conducted in a number of species. The open literature studies and unpublished study reports were peer reviewed by the International Programme on Chemical Safety (IPCS) and the conclusions of this expert group published in Environmental Health Criteria 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).



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

Section A6.2 **Annex Point IIA VI.6.2**		**Percutaneous absorption (in vivo test)** **In-vivo dermal absorption in rat dermis**	
		1 REFERENCE	**Official use only**
1.1	**Reference**	A.Th.H.J. de Bie and D. Grossouw, (2009); In vivo percutaneous absorption of an EC formulation of [^{14}C] Cypermethrin in rats. TNO Qualityof Life, Biosciences and Quality and Safety, Zeist, Netherlands. TNO report number V8114 (GLP, unpublished). Dates of experimental work: 26 January 2009 – 16 February 2009	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Agriphar S.A.	
1.2.2	Companies with letter of access		
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 for the testing of chemicals: Guideline no. 427; Skin Absorption: *in vivo* method (Paris, April 2004). OECD Environmental Health and Safety Publications, Series on Testing and Assessment no. 28. Guidance document for the conduct of skin absorption studies, Paris, March 2004. Opinion of the Scientific Committee on Plants on Commission Draft Guidance on Dermal Absorption SCP/Guide-DERM/002 (April 2002). Guidance document on Dermal Absorption (SANCO 222/2000, rev 7, 19 March 2004).	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Cypermethrin Molecular formula : $C_{22}H_{19}C_{l2}NO_3$ Molecular weight : 416.3 CAS registration number : 52315-07-08 Appearance : light brown liquid Solubility in water (pH 7) : 3.97 µg.L-1 Partition coefficient (log Pow) : 5.5 at 20°C Storage conditions : 2-10°C Supplier : Agriphar, SA Date of arrival : 24 November 2008 Expiry date : 1 June 2011 Cypermethrin 500 EC amd Cypermethrin 500EC placebo were also included in this asay. Details for these materials were:	

Section A6.2 **Annex Point IIA VI.6.2**	**Percutaneous absorption (in vivo test)** **In-vivo dermal absorption in rat dermis**	
	Cypermethrin 500 EC Lot no. : 169606 Content : 499.7 $g.L^{-1}$ Appearance : light yellowish liquid Storage conditions : 2-10°C *Supplier : Agriphar, S.A.* *Cypermethrin 500 EC placebo* Other name : DIL 203/08 AW Lot no. : BA0030/07 Content : 0 $g.L^{-1}$ Appearance : light yellowish liquid Storage conditions : 2-10°C Supplier : Agriphar, S.A.	
3.1.1 Lot/Batch number	Lot no.: TJ230054801	
3.1.2 Specification	See 3.1 above	
3.1.2.1 Description	The appearance of cypermethrin 40/60 TG was a light brown liquid (not a brown powder as described in the protocol).	
3.1.2.2 Purity	Purity : 99%	
3.1.2.3 Stability	Stable for duration of the study	
3.1.2.4 Radiolabelling	 Position of ^{14}C label [^{14}C-cyclopropane] trans Cypermethrin Lot : 07BLY094 Specific activity : 54.0 mCi/mmol Amount received : 0.5 mCi Molecular weight : 418.0 (at this specific activity) Stated radiochemical purity : >98% Storage conditions : ≤ -70°C Date of arrival : 24 November 2008 Expiry date : 24 November 2013 Supplier : Blychem Ltd., UK	
3.2 Test Animals		
3.2.1 Species	Rat	

Section A6.2 **Annex Point IIA VI.6.2**		**Percutaneous absorption (in vivo test)** **In-vivo dermal absorption in rat dermis**	
3.2.2	Strain	Wistar (Crl:(WI)WU BR)	
3.2.3	Source	Charles River Wiga GmbH, Sulzfeld, Germany	
3.2.4	Sex	Males	
3.2.5	Age/weight at study initiation	Young adults – 8 weeks old weighing approximately 250g. The rats were acclimated to the laboratory environment for at least 5 days prior to treatment. Animals were kept in a room ventilated with 9-11 air changes per hour and maintained at a temperature of 22 +/- 3°C. Relative humidity was maintained between 30-70%RH. Artificial light was on a 12h/12h light/dark daily cycle.	
3.2.6	Number of animals per group	24 males were used in the assay	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Dermal	
3.3.1	Preparation of test site	At least 20 hours prior to dosing each animal had an area of circa 20 cm^2 of skin clipped free of hair in the shoulder / dorsal region. Following clipping the area was wiped with acetone to remove fat and skin oils and then checked for abrasions. Only rats with intact skin were used for dose application. After preparation, the animals were housed in metabolism cages. Prior to dosing, a non-absorbing phyton 'O'-ring with an inside area of approximately 10 cm^2 was glued to the clipped skin using cyanoacrylate adhesive.	
3.3.2	Concentration of test substance	See Table IIIA 6.2-1 below. [^{14}C]-Cypermethrin was dissolved in acetonitrile. For dose preparation A, 7.5 MBq [^{14}C]-Cypermethrin, dissolved in acetonitrile, was transferred to a vial. Subsequently, the acetonitrile was evaporated to near-dryness under nitrogen gas, and 3.0 mL Cypermethrin 500 g/L EC (see above) was added. For dose preparation B, 5.97 MBq [^{14}C]-Cypermethrin dissolved in acetonitrile, was transferred to a vial. Subsequently, the acetonitrile was evaporated to near-dryness under nitrogen gas, and 100 µL of Cypermethin 500 g/L EC placebo, 40 times diluted with demineralised water, was added. The formulation was sonicated for about 30 minutes. Then 9.90 mL demineralised water was added while vortexing the formulation. The preparations were kept shielded from light in ambient temperature storage until required. For Part A the nominal concentration was 500 g/L and for part B the nominal concentration was 25 mg/L.	

Section A6.2 **Annex Point IIA VI.6.2**		**Percutaneous absorption (in vivo test)** **In-vivo dermal absorption in rat dermis**	
3.3.3	Specific activity of test substance	Prior to the start of the study, the radiochemical purity of [^{14}C]-Cypermethrin was verified with radio-HPLC analysis. Cypermethrin 40/60 TG was used to confirm the identity of the radiolabelled Cypermethrin. In addition, the radiochemical purity of [^{14}C]-Cypermethrin in the dose preparations was checked before each application. The specific activity of the ^{14}C- *trans*-cypermethrin supplied was 54.0 mCi/mmol.	
3.3.4	Volume applied	Dermal application onto a dorsal area of about 10 cm^2, limited by an 'O'-ring under semi-occlusive conditions. Group A A dose of 100 µL formulated [^{14}C]-Cypermethrin at a nominal concentration of 500 g/L corresponding to 5000 µg/cm^2. Each rat received a nominal radioactive dose of 250 kBq. Group B A dose of 100 µL formulated [^{14}C]-Cypermethrin at a nominal concentration of 0.25 mg/L corresponding to 0.25 µg/cm^2. Each rat received a nominal radioactive dose of 12 kBq.	
3.3.5	Size of test site	10 cm^2 - defined by 'O' ring	
3.3.6	Exposure period	Experimental start 26 January 2009 Treatment AT1 - 24 h 27- 28 January 2009 Treatment AT2 - 72 h 27- 30 January 2009 Treatment AT3 - 216 h 27 January – 5 February 2009 Treatment BT1 - 24 h 3 - 4 February 2009 Treatment BT2 - 72 h 3 - 6 February 2009 Treatment BT3 - 216 h 3 – 12 February 2009 Experimental termination 16 February 2009 The dermal absorption of [14C]-Cypermethrin was investigated in two groups of male rats consisting of 3 subgroups of 4 animals each. A (high dose): undiluted concentrate B (low dose): representative field dilution Details of the test groups are presented in Table IIIA 6.2-2 below.	
3.3.7	Sampling time	8h and 24h after initiation of skin contact, then at 24 hour intervals up to 208 hours after completion of exposure	

Section A6.2 **Annex Point IIA VI.6.2**	**Percutaneous absorption (in vivo test)** **In-vivo dermal absorption in rat dermis**	
3.3.8 Samples	Skin washing – 8h after application. Urine and faeces collected at 24 h intervals to termination At sacrifice the following samples were collected:- - 'O'-ring + protective device - Skin wash at sacrifice - Surface tape strips (stratum corneum), individually sampled - Application site (tape stripped) - Skin (non treated area) - Whole Blood - Plasma - Gastro Intestinal Tract - Residual carcass Cage washes collected at end of the collection phase. The dislodgeable dose was determined after an exposure period of 8 hours. The covering tape from the protective device was removed and retained for analysis. The unabsorbed test substance was removed from the application site by washing 8 times with a mild lukewarm soap solution using cotton swabs. The moist skin area was dried with 2 additional cotton swabs. A fresh cover was applied by fitting an extra strong tape around the body and protective device. By doing this, interference of the animal with the exposure chamber and the risk of losing the 'O'-ring and protective device was prevented. The possibility of oral ingestion of the test substance was also minimised (in practice, no parts of the protective device were subsequently found in the faeces). Skin stripping – following sacrifice the skin was shaved (the skin shavings were retained for analysis) and then the skin stripping process was initiated. Skin stripping involved the application of adhesive tape for circa 5 seconds before the tape was carefully removed against the direction of hair growth. Skin stripping was continued until a 'shiny' appearance of the viable epidermis (stratum lucidum) was evident. This indicated that the stratum corneum had been removed. Usually 12-15 tape strips are sufficient to remove the stratum corneum but for calculation purposes a total of 20 tape strips were taken. Any hair removed was combined with the first tape strip and digested in 1.5 M KOH/20% ethanol in water.	

Section A6.2 **Annex Point IIA VI.6.2**	**Percutaneous absorption (in vivo test)** **In-vivo dermal absorption in rat dermis**	

Radioactivity was determined in all samples collected.

Dose preparations	Samples of the dose preparations were taken just prior to and directly after and diluted with ethanol. Duplicate aliquots were added directly to liquid scintillant (Ultima Gold™) and radioactivity measured by liquid scintillation counting. The average radioactive concentration was used to calculate the total amount of radioactivity administered with the respective dose preparation.
Liquid specimens	Aliquots of urine, cage wash, skin wash, plasma, extracts of cover and 'O'-ring were added directly to liquid scintillant (Ultima Gold™) and the radioactivity measured by liquid scintillation counting.
Skin strips	Individual skin strips were added directly to liquid scintillant (Hionic Fluor™). After 24 hours extraction the radioactivity was measured by liquid scintillation counting.
Faeces, whole blood	Radioactivity in faeces and whole blood was determined by combustion of air-dried subsamples.

Combustion analysis

Samples were combusted using a Packard Sample Oxidizer System 387. The CO2 formed was mixed with Carbosorb™ and Permafluor E™ scintillation fluid and radioactivity measured by liquid scintillation counting.

Digestion

The application site, non-treated skin, GI-tract with contents, hair and residual carcass were digested with 2 to 4 parts of 1.5 M KOH in 20% ethanol and homogenized using an Ultra-turrax. Duplicate aliquots (0.5-1.0 g) were mixed with liquid scintillant (Hionic Fluor™) and radioactivity measured by liquid scintillation counting.

Radioactivity measurements

Radioactivity in all samples was determined by Liquid Scintillation Counting (LSC) on a Tri-Carb 3100TR liquid scintillation counter using QuantaSmart™ software. All counts were converted to DPM using tSIE/AEC (transformed Spectral Index of external standards coupled to Automatic Efficiency Correction. All radio-assays of biological specimens, except skin strips, were performed in duplicate.

Background samples

Background values were measured with each sample sequence using the respective scintillation mixture without any samples. Limits of quantification for radioactivity in samples were calculated using the background + 3x standard deviation of the counting error of the background, as limit of quantification.

Section A6.2 **Annex Point IIA VI.6.2**	**Percutaneous absorption (in vivo test)** **In-vivo dermal absorption in rat dermis**	
	4 RESULTS AND DISCUSSION	
4.1 Toxic effects, clinical signs	The animals were checked for appearance and behaviour during acclimatisation, treatment and at each sampling point. No treatment or dose related signs of toxicity or unusual behaviour were observed. The body weights in the high dose groups showed a slight reduction between the study start and sacrifice timepoints. There was little variation in bodyweight among the low dose animals over the same timeframe.	

Section A6.2 **Annex Point IIA VI.6.2**	**Percutaneous absorption (in vivo test)** **In-vivo dermal absorption in rat dermis**	
4.2 Retention at application site	Mean recovery of radioactivity ranged from 99.9% to 100.2.% in the high dose groups and from 99.8 to 105.6% in the low dose groups. Individual data expressed as µg Parent Compound equivalents per gram tissue (PCE/g tissue) are presented in the report. The amount of radioactivity in the skin strips from the application site is presented graphically in Figures 1 and 2. The amounts remaining in the application site are presented graphically in Figure 3 **Skin stripping** After the 8 h exposure and 16 h post application period – (i.e. at 24 hours after initial of dosing), 7.93% of the dose remained on/in the skin in the high dose group and and 8.53% remained in skin for the low dose group. The skin stripping process (total of 20 tapestrips used to removethe *stratum corneum* and reveal the *stratum lucidum*) showed that most radioactivity at the application site was in the stratum corneum, and therefore the potential for absorption was limited. The total amount of radioactivity in all 20 skin strips after 24 h was 7.11% and 7.41% of the administered dose for the high and low dose group, respectively. In the high dose group the mean radioactivity in total skin strips was 6.73% and 5.03% after 72 h and 216 h, respectively. For the low dose, residual radioactivity in total skin strips was 6.54% and 4.34% after 72 h and 216 h, respectively. At both dose levels skin stripping revealed that most radioactivity in the *stratum corneum* was concentrated in the upper layer and/or hair at 144 h after the start of application. **Stripped skin** In the high dose group 0.82% of the administered dose remained in the stripped skin after 24 h (recovered from skin wash at 8 h and 16 h post application) and radioactivity slightly increased to 1.23% after 216 hours. In the low dose group, 1.12% of applied radioactivity remained in the stripped skin after 24 h (8 h exposure +16 hours post exposure) but this decreased to 0.20% after 216 h. The levels of radioactivity found in control skin were negligible at all dose levels and time groups. **Plasma, whole blood and carcass** The highest radioactivity (0.59% of the dose) was found in the residual carcass of animals receiving the low dose, 24 h after the start of application. The levels of radioactivity in plasma and whole blood were close to or below the limit of detection in all time points in the low dose groups and all far below 1 ng/g.	

Section A6.2 **Annex Point IIA VI.6.2**	**Percutaneous absorption (in vivo test)** **In-vivo dermal absorption in rat dermis**	
4.3 Recovery of labelled compound	The dose preparations were analysed for stability and purity. The results are tabulated in Table IIIA 6.2-2 below. Mean recovery of radioactivity ranged from 99.9% to 100.2.% in the high dose groups and from 99.8 to 105.6%. in the low dose groups. [One rat, Animal No. 22, had a low skin wash value and a high amount of radioactivity in/on the ring and cover. As a consequence this animal was exposed for much longer to the test substance, leading to a high absorption value. The results for this animal were therefore omitted for calculation of mean and standard deviation.]	
4.4 Percutaneous absorption	**Absorption, excretion** Individual data are presented in the report for radioactivity in excreta, skin wash and tissues and summarised in Table IIIA 6.2-6 below. After administration of the high dose 0.98±0.82% of the dose was absorbed within 24 h. The total absorption increased to 1.09±0.15% at 72 h and 3.44±1.05% at 216 h after dosing. For the low dose 5.62±1.48 % of the dose was absorbed within 24 h. The total absorption increased slightly to 8.63±0.92% at 72 h and 11.03±1.66 at 216 h after dosing. The urinary excretion accounted for 2.26% and 8.18% of the high and low dose absorption, respectively, within 216 h. Recovery from faeces accounted for 0.38% and 2.08% of the high and low dose absorption, respectively, within 216 h. The bulk of the applied Cypermethrin applied to the skin was removed by washing. Radioactivity in the skin wash was 84-91% of the applied radioactivity for the low dose. In the high dose ca 91% of the applied radioactivity was found in the skin wash. The amounts of radioactivity found in the 'O'-ring and cover after application was considerable (6-8%)at the high dose but lower (2.0-2.5%) in the low dose. The average systemic dose over the last 24 h in each time group is presented in Table IIIA 6.2-7 below. The systemic dose was calculated as total radioactivity absorbed up to sacrifice minus total radioactivity excreted in the previous days (the cage wash included only in the 24 h time group). The results presented in the table demonstrate a low systemic dose (ca 1%) in the time groups of the high dose and a steady decrease from 5.6% after 24 h to ca 1% after 216 h in the low dose.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	This study was designed to examine the percutaneous absorption of [^{14}C]-Cypermethrin, formulated as Cypermethrin 500 g a.i./L EC, *in vivo* using rats. Cypermethrin was tested at two target concentrations: the emulsifiable concentrate (500 g a.i./L) and a representative field dilution (25 mg a.i./L). The study objectives were to determine the extent of percutaneous absorption of the compound related radioactivity, its permeation through the skin into the body, and its elimination *via* excreta following a	

Section A6.2 **Annex Point IIA VI.6.2**	**Percutaneous absorption (in vivo test)** **In-vivo dermal absorption in rat dermis**	

contact time of 8 hours.

The bioavailability of the residues remaining in/on skin after washing of the application site and the kinetics of percutaneous absorption were estimated at 3 time points, 24, 72 and 216 hours after the beginning of the 8 hour exposure time. Furthermore, the distribution of radioactivity between the upper skin layer (*stratum corneum*) and the rest of the skin was estimated.

Cypermethrin was applied at two dose levels - 500 g a.i./L and 25 mg a.i./L equating to the suspension concentrate and a representative field dilution. Subgroups were dosed for each assessment timepoint (24, 72 and 216 h). For each dose level and time point, 4 male Wistar rats were used. The rats were shaved prior to dosing, following suitable acclimation and determination that the skin was undamaged.

Test groups

Sub group	Nom. conc.	Nominal dose[1]	No. of rats	Exposure + post exposure time[2]	In-life period
AT1	500 g/L	5000 µg.cm^{-2}	4	8 h + 16 h	24 h
AT2	500 g/L	5000 µg.cm^{-2}	4	8 h + 64 h	72 h
AT3	500 g/L	5000 µg.cm^{-2}	4	8 h + 208 h	216 h
BT1	25 mg/L	0.25 µg.cm^{-2}	4	8 h + 16 h	24 h
BT2	25 mg/L	0.25 µg.cm^{-2}	4	8 h + 64 h	72 h
BT3	25 mg/L	0.25 µg.cm^{-2}	4	8 h + 208 h	216 h

100 µL of the dose preparations was applied to 10 cm^2 of clipped skin. The test site was defined by an 'O'ring stuck to the dermis and covered with a semi-permeable dressing.

Group A
A dose of 100 µL formulated [^{14}C]-Cypermethrin at a nominal concentration of 500 g/L corresponding to 5000 µg/cm^2. Each rat received a nominal radioactive dose of 250 kBq.

Group B
A dose of 100 µL formulated [^{14}C]-Cypermethrin at a nominal concentration of 0.25 mg/L corresponding to 0.25 µg/cm^2. Each rat received a nominal radioactive dose of 12 kBq.

The treated skin was washed at the completion of the 8 hour exposure period. Excreta samples were collected at 24 h intervals up to the scheduled sacrifice time (24, 72 or 216 hours). At sacrifice the following samples were collected -

'O'-ring + protective device; skin wash; surface tape strips (stratum corneum), individually sampled; application site (tape stripped); skin (non treated area); whole blood; plasma; gastrointestinal tract; residual carcass. The cage washings at the end of the collection period were also retained.

Verification of the amount of radioactivity applied to the skin was completed before application and analysis of recovery radioactivity was assessed based on the various collected samples and a mass balance completed.

Determination of the degree of dislodgeable dose and also the amount of

Section A6.2 **Annex Point IIA VI.6.2**	**Percutaneous absorption (in vivo test)** **In-vivo dermal absorption in rat dermis**	
	dose retained in the skin was investigated using multiple washing to remove non-absorbed material at the end of the exposure period and the use of tape strips (20 in total) to remove the *stratum corneum* sequentially until the *stratum lucidum* was revealed. Samples were analysed for radioactivity by means of liquid scintillation counting following appropriate preparation of the samples.	
5.2 Results and discussion	***Absorption (systemically available)*** Total absorption was assessed from recoveries of radioactivity in urine and faecal samples, cage wash, tissues, GI tract and residual carcass as 0.98±0.82% of the high dose absorbed within 24 h. The total absorption increased to 1.09±0.15% at 72 h and to 3.44±1.05% at 216 h after dosing. For the low dose 5.62±1.48% of the dose was absorbed within 24 h. The total absorption increased to 8.63±0.92% at 72 h and to 11.03±1.66 at 216 h after dosing. The systemic dose per day showed a low systemic dose (ca 1%) in the time groups of the high dose and a steady decrease from 5.6% after 24 h to ca 1% after 216 h in the low dose. The highest amount of radioactivity, 0.59% of the administered dose, was the residual amount found in the low dose carcases, at the 24 h sacrifice. Levels in blood and plasma were close to or below the limit of detection. The low dose values were considerably less than 1 ng/g tissue. ***Skin strips*** In the high dose mean radioactivity in skin strips was 7.11% after 24 h, 6.73% after 72 hours and 5.03% at 216 h after dosing. For the low dose, residual radioactivity in skin strips was 7.41% after 24 h, 6.54% after 72 hours and 4.34% after 216 h. These values include all 20 tape strip amounts. Due to the persistence of absorption over the extended observation period and in accordance with current guidance on use of tape strip data (eg EFSA or MOTA 3), the total amount of absorbed material or biologically available material for absorption was adjusted to exclude the amounts recovered at each time point in the first two tape strips – see discussion below. At both dose levels skin stripping revealed that most radioactivity in the stratum corneum was concentrated in the upper layer and/or hair at 216 h after the start of application and therefore the potential for further absorption was limited.	

Section A6.2 **Annex Point IIA VI.6.2**	**Percutaneous absorption (in vivo test)** **In-vivo dermal absorption in rat dermis**	
	Stripped skin Radioactivity in the dermis (stripped skin) increased from 0.82% at 24 h to 1.23% at 216 h after application in the high dose group. For the low dose the radioactivity decreased from 1.12% at 24 h to 0.20% at 216 h after application. In the high dose group sacrificed 16 hours after the 8 hour exposure, 0.82% of administered dose was found in the skin (after washing the dislodgeable amount and removing the stratum corneum by tape stripping). This residual amount in the skin increased slightly to 1.23% of administered dose when assessed at 216 hours. For the low dose groups, radioactivity in stripped skin was 1.12% of administered dose after 24 h and decreased to just 0.20% by 216 hours. ***Elimination*** The majority of the radioactivity associated with administered Cypermethrin was removed by washing. The dislodgeable, non – absorbed radioactivity in the skin wash was 84-91% of the applied radioactivity for the low dose and in the high dose circa 91% of the applied radioactivity was removed in the skin wash. Additionally significant amounts of radioactivity were associated with the 'O'-ring and cover - 6-8% in the high dose groupand slightly lower, 2.0-2.5%, in the low dose. Urinary excretion accounted for 2.26% and 8.18% of the high and low dose, respectively, measured over the 216 h assessment period. Urinary excretion at 24 and 72 hours equated to 0.28% and 0.71% of the administered dose in the high dose group and 2.74% and 6.43% in the low dose. Faecal excretion accounted for small amounts of radioactivity - 0.38% and 2.08% of the administered high and low doses respectively, within 216 h. High dose faecal excretion at 24 or 72h was 0.07% and 0.15%. The values forthe low dose group were 0.38% and 1.12% respectively.	

Section A6.2 **Annex Point IIA VI.6.2**	**Percutaneous absorption (in vivo test)** **In-vivo dermal absorption in rat dermis**	
Discussion	Based on the results of this study and taking account of the Manual of Technical Agreements (MOTA3) Biocides Technical Meeting, 24 Feb, 2010, the RMS considered *in vivo* percutaneous absorption study of an EC formulation in rats (concentrate 500g ai/L; spray dilution 25 mg a.i./L = agriculture field dilution, 8 h exposure, post exposure times 24h, 72h, 216h), revealed dermal absorption values, including residual skin (stripped skin) at 24h, 72h or 216 h after dosing of - Concentrate: 1.8, 2.1, 4.7% Spray dilution: 6.5, 9.2, 11.2%. This would assume excluding all of the tape stripping recovered radioactivity. The dermal absorption values including residual skin and **all 20** tapestrips at 24h, 72h or 216 h after dosing: Concentrate: 8.9, 8.9, 9.7% Spray dilution: 13.94, 15.8, 15.6%. The draft 'Guidance on Dermal Absorption' recently circulated by EFSA Panel on Plant Protection Products and their Residues (PPR) seeks to clarify the use of data obtained by skin stripping. In the current study, a total of 20 strips were used and the total dermal absorption values calculated above reflect either the inclusion of results from all strips or from none. Neither position is thought to accurately reflect the role of the stratum corneum as a partial barrier to dermal absorption and a means of removal of non-absorbable material. The EFSA Guidance reflects the conclusions of MOTA3 in the Biocides Technical Meeting. Where there is no evidence for continuing absorption at the end of the observation period, it is justified to exclude the tape strip data entirely. In cases where more than 75% of absorption occurs within half of the observation period, then tape strip data may alsobe excluded unless only pooled data are available. In this study the absorption after 72 hours was less than 75% of the total after 216 h and so inclusion of some tape strip data is considered appropriate. The RMS has suggested not including values equivalent to the top 25% of the stratum corneum, but in the absence of data relating to the percent of corneum removed by each strip and without an assumption of a linear dispersal of radioactivity throughout the stratum corneum it is not possible to estimate this value from the available data. The total absorption has therefore been recalculated to include the total absorbed plus any radioactivity recovered from stripped skin and also a value for the percent of applied dose recovered in tape strips 3 to 20, excluding the amounts in strips 1 and 2. These values are presented in Table IIIA 6.2-8. There is general agreement, reflected in both MOTA3 (4.1.1 Q2) and the most recent EFSA Guidance on Dermal Absorption (point 5.1.1) that the amount of dose removed by the initial two tapestrips represents material that will not become biologically available and that practically and pragmatically these data can be excluded for the total absorption calculation. Based on these assumptions the dermal absorption values for cypermethrin including total absorbed, residual skin absorption and 18 tapestrips (first two excluded) at 24 h, 72 h or 216 h after dosing are: **Concentrate: 6.7, 7.0, 7.6%** **Spray dilution: 12.5, 13.6, 12.7%.**	

Section A6.2 **Annex Point IIA VI.6.2**	**Percutaneous absorption (in vivo test)** **In-vivo dermal absorption in rat dermis**	
5.3 Conclusion	Cypermethrin formulated as 500 EC (500 g a.i./L) and a representative field dilution (25 mg a.i./L) were applied to rats for an 8 h exposure period. The majority of radioactivity associated with Cypermethrin applied to the skin was removed by washing at the end of the exposure period. Radioactivity recovered in the skin wash accounted for 84-91% of the applied radioactivity for the low dose and in the high dose circa 91% of the applied radioactivity was recovered in the skin wash. Additionally the amounts of radioactivity found in the 'O'-ring and cover after application was considerable (6-8%) in the high dose but much lower (2.0-2.5%) in the low dose. Within 24h, 0.98±0.82% of the high dose was absorbed. The total absorption increased to 1.09±0.15% at 72 h and 3.44±1.05% at 216 h after dosing. For the low dose 5.62±1.48 % of the dose was absorbed within 24 h. The total absorption increased to 8.63±0.92% at 72 h and 11.03±1.66 at 216 h after dosing. In the high dose mean radioactivity in thetotal (20) skin strips was 7.11% after 24 h and 5.03% at 216 h after dosing. For the low dose, residual radioactivity in twenty skin strips was 7.41% after 24 h and 4.34% after 216 h. Most radioactivity in the stratum corneum was concentrated in the upper layer and/or hair at 216 h after the start of application and therefore the potential for absorption was limited. Radioactivity in the dermis (stripped skin) slightly increased from 0.82% at 24 h to 1.23% at 216 h after application in the high dose group. For the low dose the radioactivity decreased from 1.12% at 24 h to 0.20% at 216 h after application. The urinary excretion accounted for 2.26% and 8.18% of the high and low dose, respectively, within 216 h. The faeces accounted for 0.38% and 2.08% of the high and low dose, respectively, within 216 h. Penetration of Cypermethrin through rat skin at a typical concentration (25 mg/L) used in the field is 11% and 3.4% for the formulated concentrate (500 g/L). There is general agreement, reflected in both MOTA3 (4.1.1 Q2) and the most recent EFSA Guidance on Dermal Absorption (point 5.1.1) that the amount of dose removed by the initial two tapestrips represents material that will not become biologically available and that practically and pragmatically these data can be excluded for the total absorption calculation. Based on these assumptions the **revised** dermal absorption values for cypermethrin including total absorbed, residual skin absorption and 18 tapestrips (first two excluded) at 24 h, 72 h or 216 h after dosing are: **Concentrate: 6.7, 7.0, 7.6%** **Spray dilution: 12.5, 13.6, 12.7%.**	
5.3.1 Reliability	1	
5.3.2 Deficiencies	No	

Section A6.2 **Annex Point IIA VI.6.2**	**Percutaneous absorption (in vivo test)** **In-vivo dermal absorption in rat dermis**	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	January, 2011.	
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	1	
Acceptability	acceptable	
Remarks		
	COMMENTS FROM ...	
Date		
Materials and Methods		
Results and discussion		
Conclusion		
Reliability		
Acceptability		
Remarks		

Table IIIA 6.2-1 Dose preparations

Group	Dose	[^{14}C]-Cypermethrin	Cypermethrin 500 g/L EC	Cypermethrin 500 g/L EC placebo	Nominal concentration	Nominal radioactivity concentration
A	High dose	7.50 MBq (~ 1.57 mg)	3.0 mL	-	500 g.L^{-1}	2.5 MBq.mL^{-1}
B	Low dose	5.97 MBq (~1.25 mg)	-	50 mL*	25 mg.L^{-1}	120 kBq.mL^{-1}

- diluted 20000 times with demineralised water
-

Table IIIA 6.2-2 Characteristics of dose preparations

Dose preparation (day of use)	Concentration		Specific Activity ** (kBq/mg a.i.)	Purity HPLC (%)***
	DPM/g ± CV	mg a.i.*/g		
AT1, AT2, AT3 (27 January 2009)	1.436E+08 ± 0.19%	478.77	4.999	96.9-96.8
BT1, BT2, BT3 (3 February 2009)	6.913E+06 ± 4.42%	0.0241	4780	97.1-96.9

* Active ingredient.

** Calculated by total radioactivity measured divided by total labelled and non-labelled Cypermethrin.

*** The purity before and directly after dosing is given

Table IIIA 6.2-3 Overview of test groups

Subgroup	Nominal concentration	Nominal dose[1]	Number of animals	Exposure/post exposure time[2]	In-life period
AT1	500 g.L^{-1}	5000 µg.cm^{-2}	4	8 h + 16 h	24 h
AT2	500 g.L^{-1}	5000 µg.cm^{-2}	4	8 h + 64 h	72 h
AT3	500 g.L^{-1}	5000 µg.cm^{-2}	4	8 h + 208 h	216 h
BT1	25 mg.L^{-1}	0.25 µg.cm^{-2}	4	8 h + 16 h	24 h
BT2	25 mg.L^{-1}	0.25 µg.cm^{-2}	4	8 h + 64 h	72 h
BT3	25 mg.L^{-1}	0.25 µg.cm^{-2}	4	8 h + 208 h	216 h

1 100 µL of the dose preparations was applied to 10 cm^2 of clipped skin.

2 post-exposure monitoring after washing skin with a neutral aqueous detergent to remove non-absorbed material.

Calculations performed on experimental data

All calculations were performed with electronic devices with varying degrees of soft- and hardware dependent floating-point precision. Numerical values in this report are frequently rounded to a smaller number of digits than were used in the actual calculation to increase readability and to indicate the approximate precision of the reported results. Minor differences in results obtained from calculations with such rounded values in comparison to those obtained with higher precision values are well within the limits of the experimental accuracy and therefore of no practical concern. Data resulting in 0.0 or 0.00 after rounding are reported as < 0.1 and < 0.01, respectively. Non-rounded data were used for calculating means and standard deviations.

Activity dosed per animal:

TC = dose * TCG - dose rest

where TC = total DPM administered to the animal
dose = weight of the administered dose in g
TCG = radioactivity of the administered dose in DPM/g
dose rest = total DPM in the dose rest

Radioactivity calculated per gram of sample:

XC(1, 2) = (XD(1, 2) – Blank) / XW(1, 2)
XC = (XC(1) + XC(2)) / 2

where XC = concentration in DPM per g of sample X
XD = DPM counted for aliquot of sample X
XW = weight of aliquot of sample X
(1, 2) = various aliquots
Blank = appropriate blank value in DPM

Radioactivity calculated in µg parent compound equivalents per g of tissue:

XPC = XC / SA

where XPC = concentration in µg parent compound equivalents per g of sample X
XC = concentration in DPM per g of sample X
SA = specific activity of the test substance in DPM per µg of parent compound

Radioactivity calculated as percentage of the dose administered:

X% = (XC * X / TC) * 100

where X% = percentage radioactivity of the dose administered in sample X
XC = concentration in DPM per g of sample X
X = total weight of sample X in g
TC = total DPM administered to the animal

All results were rounded to two digits. If the result was lower than 0.005% the result is presented as < 0.01.

Limit of Quantification (µg/g):

LQ(PCE) = Blank/XW * SA

where LQ(PCE) = Limit of determination of sample in µg Parent Compound Equivalents/g
Blank = appropriate blank value in DPM
XW = Weight of aliquot of sample X in g
SA = Specific Activity of the test substance (DPM/µg Parent Compound Equivalents)

	Absorbed fraction	µg equivalents in carcass plus sum of the tissues and organs
	(systemically available)	prepared plus blood, urine, cage wash, and faeces
	Systemic dose	% of radioactivity absorbed in the body (excluding application site) up to sacrifice minus % of radioactivity excreted in the previous days
	Amounts on/in skin	µg equivalents on/in skin and tape strips
	Not absorbed fraction	µg equivalents in the skin wash, and on the protective device

Table IIIA 6.2-4 Summary table of systemic dose

Systemic dose (expressed as % of administered dose)						
Nominal dose	A: 5000 µg.cm^{-2}			B: 0.25 µg.cm^{-2}		
Time group	T1	T2	T3	T1	T2	T3
period	0-24 h	48-72 h	192-216 h	0-24 h	48-72 h	192-216 h
Systemic dose	0.98 ± 0.82	0.49 ± 0.18	1.04 ± 0.72	5.62 ± 1.48	2.21 ± 0.19	1.06 ± 0.20

Table IIIA 6.2-5 Concentration and distribution of Cypermethrin in tissue

Average values (n =4) expressed as µg PCE/ g of tissue*						
Nominal dose	A: 5000 µg.cm^{-2}			B: 0.25 µg.cm^{-2}		
Time	T1	T2	T3	T1	T2	T3
group	24 h	72 h	216 h	0-24 h	71 h	216 h
Plasma	0.182	0.130	0.190	0.00007	0.00005	< 0.00002
Whole Blood	0.068	0.135	0.102	0.00015	0.00008	0.00004
Residual Carcass	2.436	0.418	2.463	0.00007	0.00006	<0.00005

*µg Parent compound Equivalents per gram of tissue

Figure 1

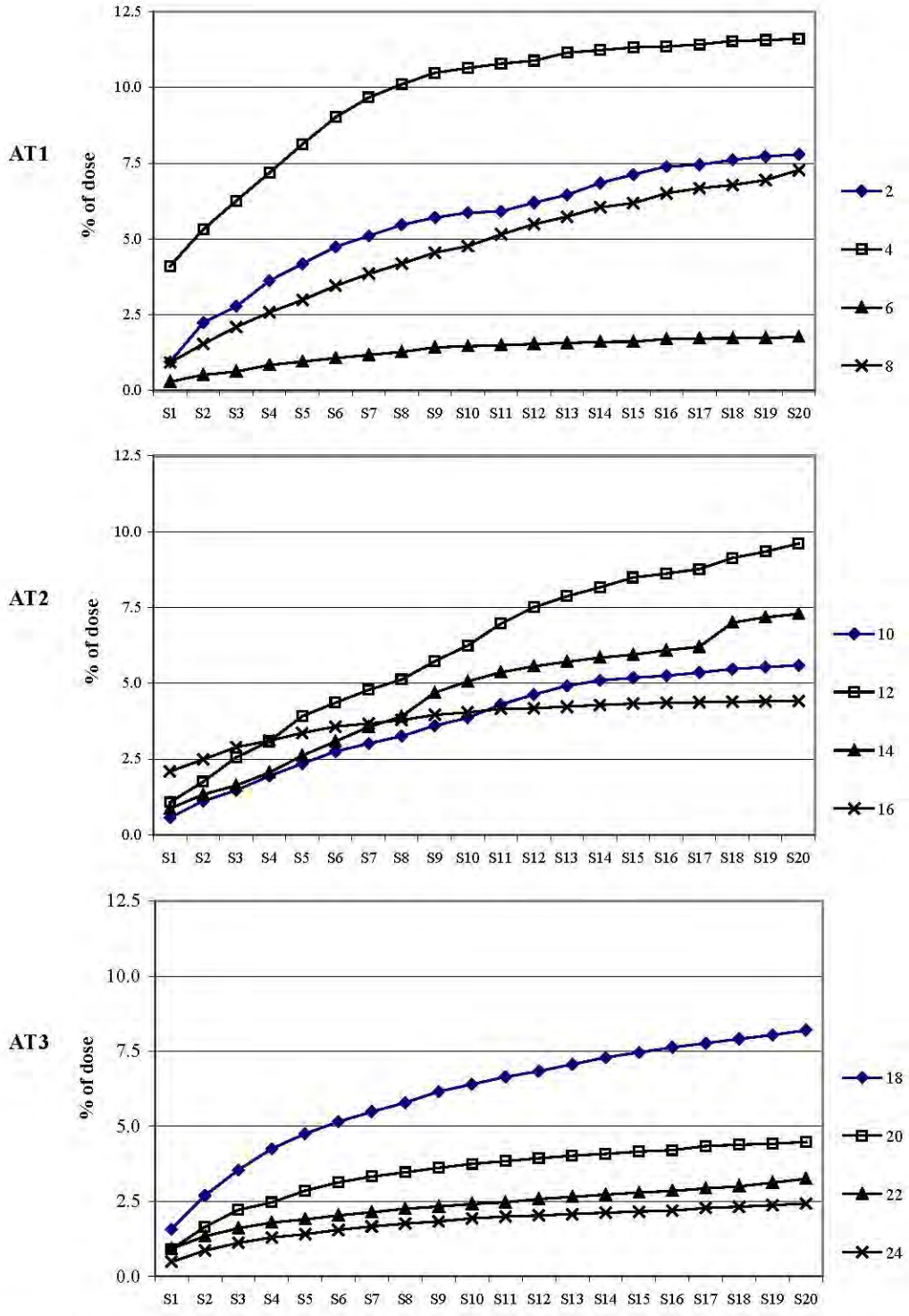


Figure A4 *Skin stripping of the application site of male Wistar rats,16 h (AT1), 64 h (AT2) or 216 h (AT3) after removal of the unabsorbed dose following dermal application of a high dose (A: 5000 µg/cm^2) of [^{14}C]-Cypermethrin*
Expressed as cumulative % of the dose stripped.
Strip number S1-S20, the legend show the individual rat numbers

Figure 2

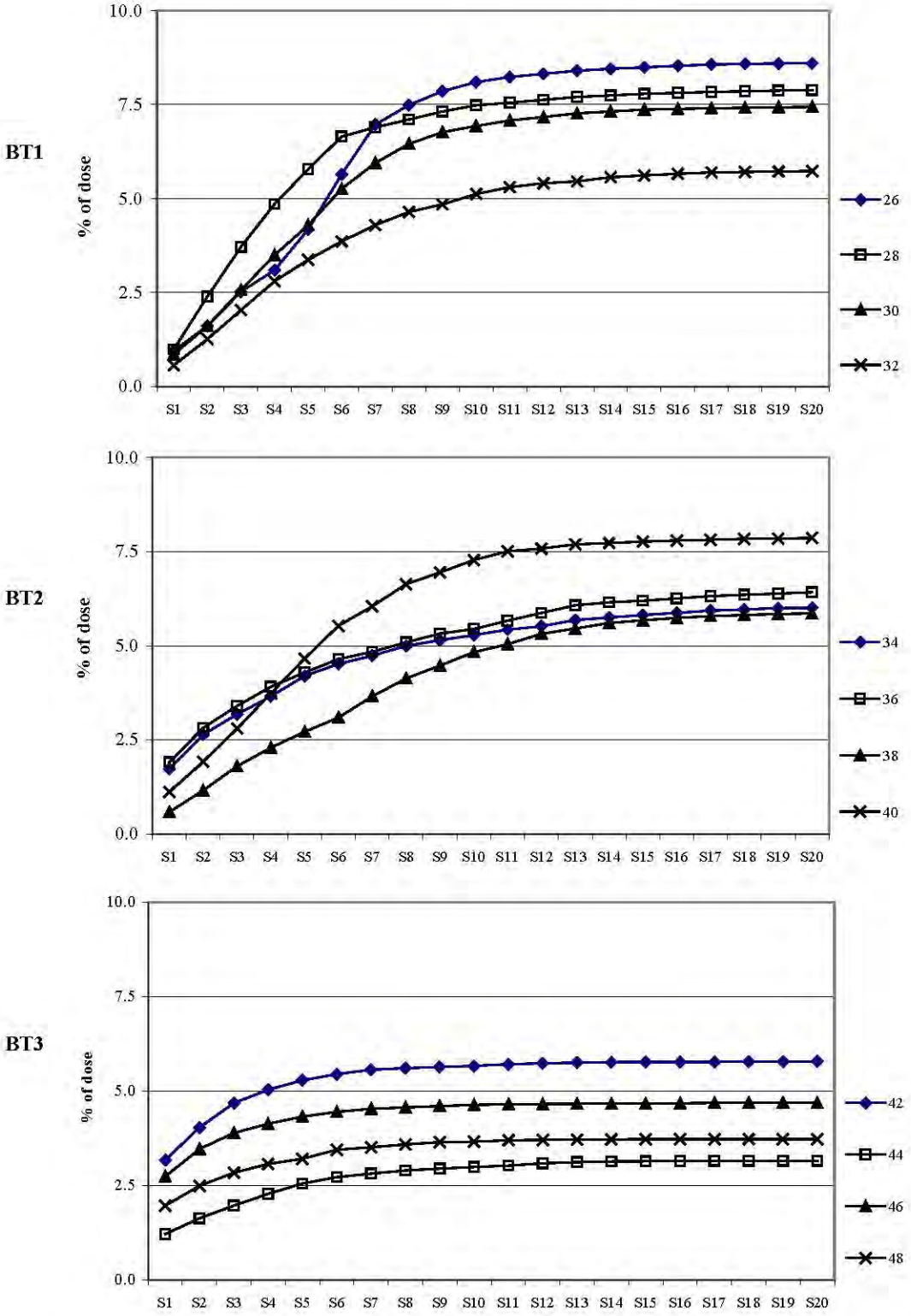
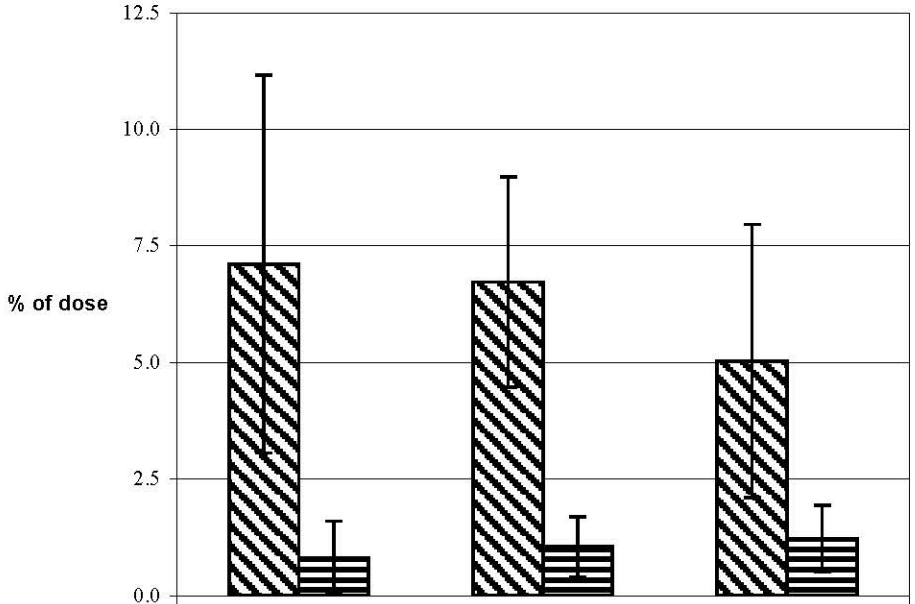


Figure A5 *Skin stripping of the application site of male Wistar rats,16 h (BT1), 64 h (BT2) or* 216 h (BT3) after removal of the unabsorbed dose following dermal application of a low dose (B: 0.25 µg/cm^2) of [^{14}C]-Cypermethrin. Expressed as cumulative % of the dose stripped. *Strip number S1-S20, the legend show the individual rat numbers.*

Figure 3

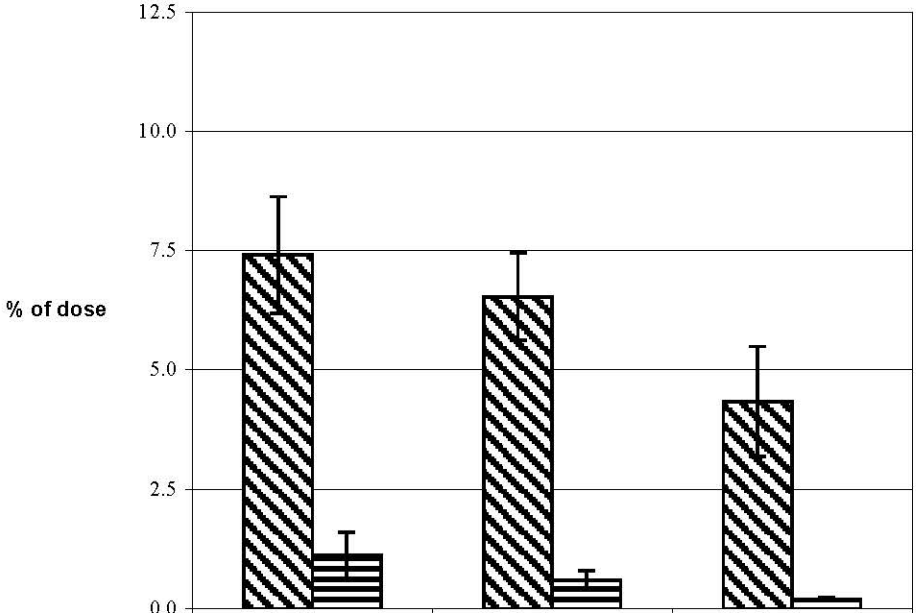
Distribution of radioactivity in skin strips and stripped skin in the application site of male Wistar rats 16 h (T1), 36 h (T2) or 136 h (T3)after removal of the unabsorbed dose following dermal application of a high dose (A: 5000 µg/cm^2) or a low dose (B: 0.25µg/cm^2) of [^{14}C]-Cypermethrin.
Expressed as % of the dose (average ± SD).

A



	AT1	AT2	AT3
Skin strips	7.11	6.73	5.03
Stripped skin	0.82	1.05	1.23

B



	BT1	BT2	BT3
Skin strips	7.41	6.54	4.34
Stripped skin	1.12	0.60	0.20

Table IIIA 6.2-6 Summary table of absorption and excretion

Average values (n=4) expressed as % of the dose applied						
Nominal dose	A: 5000 µg.cm^{-2}			B: 0.25 µg.cm^{-2}		
Time group	T1	T2	T3	T1	T2	T3
	24 h	72 h	216 h	24 h	72 h	216 h
Urine (total)	0.28	0.71	2.26	2.74	6.43	8.18
Faeces (total)	0.07	0.15	0.38	0.38	1.12	2.08
Cage wash	0.01	0.01	0.09	0.18	0.24	0.12
Tissues**	< 0.01	< 0.01	< 0.01	0.06	0.04	0.05
GI-tract	0.13	0.07	0.15	1.66	0.29	0.17
Carcass	0.49	0.15	0.56	0.59	0.51	< 0.43
Absorbed	**0.98**	**1.09**	**3.44**	**5.62**	**8.63**	**11.03**
Skin strips	7.11	6.73	5.03	7.41	6.54	4.34
Stripped skin	0.82	1.05	1.23	1.12	0.60	0.20
Total skin	**7.93**	**7.78**	**6.26**	**8.53**	**7.13**	**4.54**
Total skinwash	82.75	84.80	82.18	89.53	85.43	81.76
O-ring/cover	8.38	6.26	8.34	1.95	2.46	2.42
Not absorbed	**91.13**	**91.06**	**90.52**	**91.48**	**87.89**	**84.18**
Recovery	**100.04**	**99.93**	**100.22**	**105.64**	**103.66**	**99.75**

** : Terminal blood obtained by exsanguination + control skin

Table IIIA 6.2-7 Summary table of systemic dose

Systemic dose (expressed as % of administered dose)						
Nominal dose	A: 5000 µg.cm^{-2}			B: 0.25 µg.cm^{-2}		
Time group	T1	T2	T3	T1	T2	T3
period	0-24 h	48-72 h	192-216 h	0-24 h	48-72 h	192-216 h
Systemic	0.98	0.49	1.04	5.62	2.21	1.06
dose	± 0.82	± 0.18	± 0.72	± 1.48	± 0.19	± 0.20

Table IIIA 6.2-8 Revised estimate of dermal absorption (not including amounts recovered in first two tape strips)

Average values (n=4) expressed as % of the dose applied						
Nominal dose	A: 5000 µg.cm^{-2}			B: 0.25 µg.cm^{-2}		
Time group	T1	T2	T3	T1	T2	T3
	24 h	72 h	216 h	24 h	72 h	216 h
Absorbed (Sum of urine, faeces, cage wash, tissues, GI tract and residual carcass	**0.98**	**1.09**	**3.44**	**5.62**	**8.63**	**11.03**
Skin strips All 20 included	7.11	6.73	5.03	7.41	6.54	4.34
Stripped skin	0.82	1.05	1.23	1.12	0.60	0.20
Total skin	**7.93**	**7.78**	**6.26**	**8.53**	**7.13**	**4.54**
Skin strips, first two strips removed	4.89	4.83	2.89	5.77	4.38	1.48
Stripped skin	0.82	1.05	1.23	1.12	0.60	0.20
Total skin	**5.70**	**5.90**	**4.12**	**6.89**	**4.98**	**1.68**
Total absorption	**6.68**	**6.99**	**7.56**	**12.51**	**13.61**	**12.71**

Section A6.3.1 (01) Repeated dose toxicity

Annex Point IIA 6.3.1

Short-term repeated dose toxicity (oral) - Rat

			Official use only
		1 REFERENCE	
1.1	**Reference**	Coombs, A.D., Carter, B.I., Hend R.W., Buterworth S.G., Buckwell, A.C. (1976); Toxicity studies on the insecticide WL 43467 (cypermethrin): summary of results of preliminary experiments; Shell UK Ltd., report no. TLGR.0104.76 (CYP/T2), 1976 (unpublished).	
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**	No	
		Existing study comparable to method B7 of Directive 92/69/EEC	
2.2	**GLP**	No	
		GLP was not compulsory at the time the study was performed	
2.3	**Deviations**	Yes	
		Limited enquiries compared to a more modern study, no raw data included in report.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	WL 43467 (cypermethrin)	
		1:1 mixture of cis/trans isomers	
3.1.1	Lot/Batch number	19	
3.1.2	Specification	Deviating from specification given in section 2 as follows	
3.1.2.1	Description	Viscous liquid	
3.1.2.2	Purity	unknown	
3.1.2.3	Stability	Stable	X
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Not specified	
3.2.3	Source	Charles River Ltd., Kent	
3.2.4	Sex	Males and females	
3.2.5	Age/weight at study initiation	5 weeks	
3.2.6	Number of animals per group	6 rats per sex, per group	
3.2.7	Control animals	Yes	

Section A6.3.1 (01) Repeated dose toxicity

Annex Point IIA 6.3.1 **Short-term repeated dose toxicity (oral) - Rat**

3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of treatment	5 weeks	
3.3.2	Frequency of exposure	Not specified in report, dietary exposure.	
3.3.3	Postexposure period	None	
3.3.4	**Oral**		
3.3.4.1	Type	In diet	
3.3.4.2	Concentration	1.25, 5, 12.5, 37.5, 75 mg/kg /d (25, 100, 250, 750, 1500 ppm)	X
3.3.4.3	Vehicle	Not mentioned in report	
3.3.4.4	Concentration in vehicle	Not mentioned in report	
3.3.4.5	Total volume applied	Not mentioned in report	
3.3.4.6	Controls	Fed control diet only	
3.4	**Examinations**		
3.4.1	Observations		
3.4.1.1	Clinical signs	Yes	
3.4.1.2	Mortality	Yes	
3.4.2	Body weight	Yes (recorded weekly)	
3.4.3	Food consumption	Yes (recorded weekly)	
3.4.4	Water consumption	No	
3.4.5	Ophthalmoscopic examination	No	
3.4.6	Haematology	Yes (at necropsy) Packed cell volume, haemoglobin, erythrocyte count, leucocyte count, kaolin-cephalin coagulation time, prothombin time, differential leucocyte count	
3.4.7	Clinical Chemistry	Yes (at necropsy) Protein, urea, plasma alkaline phosphatase, plasma glutamic pyruvic transaminase, plasma glutamic oxaloacetic transaminase, sodium, potassium, chloride	
3.4.8	Urinalysis	No	
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	Yes Organs: liver, kidneys, testes, spleen, brain, heart	

Section A6.3.1 (01) **Repeated dose toxicity**

Annex Point IIA 6.3.1 **Short-term repeated dose toxicity (oral) - Rat**

3.5.2	Gross and histopathology	Yes Organs: brain, heart, kidney, lung, spleen, liver , oesophagus, stomach, intestine (small and large), urinary bladder, pancreas, salivary gland, thymus, lymph nodes, gonads, prostate or uterus, pituitary, adrenals, thyroid, eye, and peripheral nerve.
3.5.3	Other examinations	No
3.5.4	Statistics	Yes. Results were expressed as statistically significant but no further details of statistical methods detailed in the report.
3.6	**Further remarks**	
	4 RESULTS AND DISCUSSION	
4.1	**Observations**	
4.1.1	Clinical signs	Four out of 6 males and one out of six females fed 1500ppm cypermethrin (the top dose) exhibited piloerection, nervousness and uncoordinated movement from week 2 onwards. No signs of pyrethroid intoxication were seen in any other dose group.
4.1.2	Mortality	No mortalities recorded during the study.
4.2	**Body weight gain**	At 1500ppm, body weight gain and terminal body weight were reduced for both male and female rats.
4.3	**Food consumption and compound intake**	At 1500ppm, food intake was reduced for both male and female rats.
4.4	**Ophthalmoscopic examination**	Not performed
4.5	**Blood analysis**	
4.5.1	Haematology	Increase in hemoglobin in males in the 1500ppm group.
4.5.2	Clinical chemistry	At 1500ppm: Increase in blood urea concentration and hemoglobin in males and in plasma alkaline phosphatase activity in females
4.5.3	Urinalysis	Not performed
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	At 1500ppm : Significant increase in relative liver weight of females. A decrease in liver weight in male rats fed 250ppm cypermethrin was not considered to be of biological significance.
4.6.2	Gross and histopathology	No compound related histopathological effects at any dose level.

Section A6.3.1 (01)	**Repeated dose toxicity**	
Annex Point IIA 6.3.1	**Short-term repeated dose toxicity (oral) - Rat**	
4.7 **Other**	No other changes were observed which could be attributed to cypermethrin at any dose level.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 **Materials and methods**	6 Charles River rats/sex/group were fed a diet containing cypermethrin (unknown purity) at 25, 100 250, 750 and 1500 ppm (converted doses 1.25, 5, 12.5, 37.5, 75 mg/kg bw/d.) for 5 weeks. 14 Animals/sex were used in the control group.	
5.2 **Results and discussion**	At 1500ppm (the top dose) body weight gain, food intake and terminal body weight were all reduced for both male and female rats. Clinical signs in this dose group were piloerection, nervousness, uncoordinated movement noted in 4/6 males and 1/6 female from week 2 onwards. An increase in blood urea concentration and hemoglobin was found in males in the 1500ppm group and an increase in plasma alkaline phosphatase activity was recorded in females. Significant increase in relative liver weight of females was also observed. No other changes were seen that could be attributed to the feeding of cypermethrin. No compound related histopathological effects were found. No signs of pyrethroid intoxication were seen in either male or female rats fed cypermethrin at concentrations of 750, 250, 100, or 25 ppm.	
5.3 **Conclusion**		
5.3.1 LO(A)EL		
5.3.2 NO(A)EL	NOAEL= 750 ppm (37.5 mg/kg bw/d)	
5.3.3 Other		
5.3.4 Reliability	2	X
5.3.5 Deficiencies	Yes. Deviations from official protocol – limited findings and no raw data were included in the brief summary report. However the study data is considered useful. 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.
Materials and Methods	The applicants version is acceptable with the following amendments: 3.1.2.3. Stability: no data shown 3.3.4.2. Concentration: 25, 100, 250, 750, 1500 ppm
Results and discussion	The applicant's version is adopted.

Section A6.3.1 (01) Repeated dose toxicity

Annex Point IIA 6.3.1 **Short-term repeated dose toxicity (oral) - Rat**

Conclusion	LO(A)EL:1500 ppm NO(A)EL: 750 ppm However, this study is of poor validity: Results are only given in a (short) descriptive way. No data tables (means nor raw data) are made available.
Reliability	3
Acceptability	Acceptable, when considered as providing additional information for longer term repeated dose toxicity (e.g. 90d studies).
Remarks	

	COMMENTS FROM ... *(specify)*
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 A6.3.1 (02) Repeated dose toxicity

Annex Point IIA 6.3.1

Short-term repeated dose toxicity (oral) - Dog

			Official use only
		1 REFERENCE	
1.1	**Reference**	Coombs, A.D., Carter, B.I., Hend R.W., Buterworth S.G., Buckwell, A.C. (1976); Toxicity studies on the insecticide WL 43467 (cypermethrin): summary of results of preliminary experiments; Shell UK Ltd., report no. TLGR.0104.76 (CYP/T2), 1976 (unpublished)	
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**	No	
		Existing study comparable to method B7 of Directive 92/69/EEC.	
2.2	**GLP**	No	
		GLP was not compulsory at the time the study was performed	
2.3	**Deviations**	Yes	
		Limited enquiries compared to a more modern study, no raw data included in report.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	WL 43467 (cypermethrin)	
		1:1 mixture of cis/trans isomers	
3.1.1	Lot/Batch number	19	X
3.1.2	Specification	Deviating from specification given in section 2 as follows	
3.1.2.1	Description	Viscous liquid	
3.1.2.2	Purity	unknown	
3.1.2.3	Stability	Stable	X
3.2	**Test Animals**		
3.2.1	Species	Dog	
3.2.2	Strain	beagle	
3.2.3	Source	Not specified in report	
3.2.4	Sex	Males and females	
3.2.5	Age/weight at study initiation	Not specified in report	
3.2.6	Number of animals per group	3 dogs per sex, per group	
3.2.7	Control animals	Yes	

Section A6.3.1 (02) Repeated dose toxicity

Annex Point IIA 6.3.1 **Short-term repeated dose toxicity (oral) - Dog**

3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of treatment	5 weeks	
3.3.2	Frequency of exposure	Not specified, dietary exposure.	
3.3.3	Postexposure period	None	
3.3.4	**Oral**		
3.3.4.1	Type	In diet	
3.3.4.2	Concentration	0, 0.375, 3.75 and 37.5 mg/kg /d (0, 15, 150 and 1500 ppm)	X
3.3.4.3	Vehicle	Not mentioned in report	X
3.3.4.4	Concentration in vehicle	Not mentioned in report	
3.3.4.5	Total volume applied	Not mentioned in report	
3.3.4.6	Controls	Fed control diet only	
3.4	**Examinations**		
3.4.1	Observations		
3.4.1.1	Clinical signs	Yes (observed daily)	
3.4.1.2	Mortality	Yes (observed daily)	
3.4.2	Body weight	Yes (recorded weekly)	
3.4.3	Food consumption	Yes (observed daily)	
3.4.4	Water consumption	No	
3.4.5	Ophthalmoscopic examination	Yes, in the top dose and control group animals at the start and end of the study.	
3.4.6	Haematology	Yes (at intervals throughout the study and at the end of the study) Packed cell volume, haemoglobin, erythrocyte count, leucocyte count, kaolin-cephalin coagulation time, prothombin time, differential leucocyte count	
3.4.7	Clinical Chemisty	Yes (at intervals throughout the study and at the end of the study) Protein, urea, plasma alkaline phosphatase, plasma glutamic pyruvic transaminase, plasma glutamic oxaloacetic transaminase, sodium, potassium, chloride, glucose.	
3.4.8	Urinalysis	No	
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	Yes Organs: liver, kidneys, testes, spleen, brain, heart, thyroid.	

Section A6.3.1 (02) **Repeated dose toxicity**

Annex Point IIA 6.3.1 **Short-term repeated dose toxicity (oral) - Dog**

3.5.2	Gross and histopathology	Yes Organs: brain, heart, kidney, lung, spleen, liver , oesophagus, stomach, intestine (small and large), urinary bladder, pancreas, salivary gland, thymus, lymph nodes, gonads, prostate or uterus, pituitary, adrenals, thyroid, peripheral nerve, gall bladder, aorta.
3.5.3	Other examinations	No
3.5.4	Statistics	Yes, Results were expressed as statistically significant but no further details of statistical methods detailed in the report.
3.6	**Further remarks**	-
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	In the 1500ppm group (the top dose) animals exhibited apprehension, diarrhoea and vomiting, licking and chewing of the paws, whole body tremors and stiff exaggerated hind leg gait, and ataxia. Two animals (1 female, 1 male) convulsed during week 1 and 5 respectively. No signs of pyrethroid intoxication were seen in any other dose group.
4.1.2	Mortality	No mortalities recorded during the study.
4.2	**Body weight gain**	Significantly reduced at 1500ppm due to the observed loss of apetite exhibited by animals in this dose group.
4.3	**Food consumption and compound intake**	At 1500ppm animals exhibited a loss of apetite.
4.4	**Ophthalmoscopic examination**	No ocular abnormalities were found in the 1500ppm and control groups examined.
4.5	**Blood analysis**	
4.5.1	Haematology	In the 1500ppm group, male dogs showed an increase in WBC and Kaolin-cephalin coagulation time (KCCT) values at week 5 of the study.
4.5.2	Clinical chemistry	At 1500ppm: Female blood urea concentrations were increased and blood glucose levels decreased at week 5 of the study.
4.5.3	Urinalysis	Not performed
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	At 1500ppm : Increased relative thyroid weight in both males and females.
4.6.2	Gross and histopathology	No compound related gross pathological and histopathological effects at any dose level.

Section A6.3.1 (02)	**Repeated dose toxicity**
Annex Point IIA 6.3.1	**Short-term repeated dose toxicity (oral) - Dog**

4.7	**Other**	No other changes were observed which could be attributed to cypermethrin at any dose level.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	3 Beagle dogs/sex/group were fed a diet containing cypermethrin (unknown puritiy) at 0, 15, 150 and 1500 ppm (0.375, 3.75 and 37.5 mg/kg bw/d) for 5 weeks.
5.2	**Results and discussion**	At 1500ppm: *Body weight gain, food intake and terminal body weight*: loss of appetite, the latter accounting for a significantly decreased body weight gain . *Clinical signs*: apprehension, diarrhoea and vomiting, licking and chewing of the paws, whole body tremors and stiff exaggerated hind leg gait, ataxia . Two animals convulsed during week 1 and 5 respectively. *Haematology/Clinical chemistry*: at week 5, female blood urea levels were increased and blood glucose levels decreased. Male dogs showed an increase in WBC and Kaolin-cephalin coagulation time (KCCT) values. *Organ weight*: Male and female relative thyroid weight were increased. No other changes were seen that could be attributed to the feeding of cypermethrin. No compound related histopathological effects were found. No signs of pyrethroid intoxication were seen in either male or female dogs fed cypermethrin at concentrations of 150 or 15 ppm.
5.3	**Conclusion**	
5.3.1	LO(A)EL	
5.3.2	NO(A)EL	NOAEL= 150 ppm (3.75 mg/kg bw/d)
5.3.3	Other	
5.3.4	Reliability	2
5.3.5	Deficiencies	Yes. Deviations from official protocol – limited findings and no raw data were included in the brief summary report. However the study data is considered useful as dogs are a more sensitive species compared to rats. 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 A6.3.1 (02) Repeated dose toxicity

Annex Point IIA 6.3.1

Short-term repeated dose toxicity (oral) - Dog

Materials and Methods	The applicant's version is acceptable with the following amendments: 3.1.1. Lot/Batch: not mentioned 3.1.2.3. Stability: no data shown 3.3.4.2. Concentration: 0, 15, 150, 1500 ppm 3.3.4.3. Vehicle: aceton
Results and discussion	*The applicant's version is adopted.*
Conclusion	LO(A)EL: 1500 ppm NO(A)EL: 150 ppm However, this study is of poor validity: Results are only given in a (short) descriptive way. No data tables (means nor raw data) are made available.
Reliability	*3*
Acceptability	Acceptable, when considered as providing additional information for longer term repeated dose toxicity (e.g. 90d studies).
Remarks	

	COMMENTS FROM ... *(specify)*
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 6.3.2, 6.4.2 **Annex Point IIA 6.3, 6.4**	**Repeated Dose / Subchronic Toxicity (Dermal)**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [√]**	
Limited exposure [√]	**Other justification [ ]**	
Detailed justification:	Data is available from repeated dose studies showing cypermethrin is of low toxicity when administered via the oral route: 5 week study in rat: NOAEL= 750 ppm =37.5 mg/kg bw 90 day study in rat: NOAEL=100 ppm = 5 mg/kg bw (later updated to 20 mg/kg by the ECCO review under Directive 91/414/EC) 90 day study in dog: NOAEL =500 ppm = 12.5 mg/kg bw In terms of dermal exposure, the worst case scenario would be the use of cypermethrin for dipping treatments (PT8). However actual workplace exposure would be low due to the requirement for PPE (coveralls and gloves) to be worn by workers in the dipping plant. In addition, a typical industrial dipping plant will employ a high degree of automation to ensure limited contact with the product and also the freshly treated wood. Dipping is done mechanically and is operated from a control panel, with wood loaded into a cradle/frame before being lowered into solution. In most plants wood is loaded and unloaded using forklift trucks. Vacuum-pressure and industrial spraying treatments both employ a closed system. Again PPE and engineering controls will be in place to prevent worker exposure. For other superficial treatments the concentration of cypermethrin used is relatively low (0.01-1.0% depending on the target insect and the type of formulation). Furthermore there will be little or no repeated exposure as treatments are made relatively infrequently. This is particularly important when considering amateur use where PPE is not always worn. In this case the user will most likely be applying the product only once every few years. A dermal absorption study has been performed using a 10% EC Cypermethrin formulation (worst case for dermal uptake). The test was performed using two dose levels, 10% and 0.0025% cypermethrin (see Document IIIA_6.2-02). A dermal absorption of 1.5% was assigned for the 10% undiluted formulation and a dermal absorption of 13% for the 0.0025% diluted formulation. In the risk assessment for PT 8 (Document IIB and IIC), the potential dermal exposures for each type of treatment application has been estimated using the TNsG exposure scenario for water based wood preservatives (Handling Model 1) and taking into account the experimental dermal absorption for cypermethrin of 13%. These exposure values have been used to calculate the Acceptable Operator Exposure Level (AOEL) using the NOAEL of 12.5 mg/kg bw/day from the subchronic oral study in the dog and applying an assessment factor of 100. The AOEL value is adjusted to allow for absorption of 47.3% seen in the rat ADE study (Document IIIA_6.2_01) to give a working AOEL of 0.059 mg/kg.	

Section 6.3.2, 6.4.2 **Annex Point IIA 6.3, 6.4**	**Repeated Dose / Subchronic Toxicity (Dermal)**

Percentage AOEL values can then be calculated for each of the exposure scenarios and are summarised in the table below.

Exposure scenario	Systemic dose (mg/kg bw/d)		AOEL	% AOEL	
	95th percentile	50th percentile		95th percentile	50th percentile
Vac-pressure	0.00330	0.000430	0.059	5.08	0.73
Dipping	0.0022	0.00034	0.059	0.013	0.576
Spraying cabinets	Very low (EASE)		0.059	<<100%	

Therefore it can be concluded that there is no concern for workers in terms of dermal exposure to cypermethrin. Taking into account the use of PPE and therefore the limited exposure, and in the interest of minimising unnecessary animal testing, further repeated dose/subchronic studies via the dermal route are not considered necessary.

Undertaking of intended data submission [ ]	Not applicable

Evaluation by Competent Authorities

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

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	April, 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 6.3.3, 6.4.3
Annex Point IIA 6.3, 6.4

Repeated Dose / Subchronic Toxicity (Inhalation)

JUSTIFICATION FOR NON-SUBMISSION OF DATA — Official use only

Other existing data [ ] **Technically not feasible** [ ] **Scientifically unjustified** [√]

Limited exposure [√] **Other justification** [ ]

Detailed justification:

Based on the very low vapour pressure, there is no concern for workers from inhalation exposure as levels of cypermethrin in the atmosphere will be low.

Data is available from repeated dose studies showing cypermethrin is of low toxicity when administered via the oral route:

5 week study in rat: NOAEL= 750 ppm =37.5 mg/kg bw

90 day study in rat: NOAEL=100 ppm = 5 mg/kg bw (later updated to 20 mg/kg by the ECCO review under Directive 91/414/EC)

90 day study in dog: NOAEL =500 ppm = 12.5 mg/kg bw

In terms of inhalation exposure, actual workplace exposure would be low due to the recommended requirement for PPE to be worn by industrial operators applying the formulated product. The only spray application is performed on an industrial scale in enclosed cabinets, therefore worker inhalation exposure is not an issue.

In the risk assessment for PT 8 (Document IIB and IIC), the potential inhalation exposures for each type of treatment application has been estimated using the TNsG exposure scenario for water based wood preservatives (Handling Model 1). These exposure values have been used to calculate the Acceptable Operator Exposure Level (AOEL) using the NOAEL of 12.5 mg/kg bw/day from the subchronic oral study in the dog and applying an assessment factor of 100. The AOEL value is adjusted to allow for absorption of 47.3% seen in the rat ADE study (Document IIIA_6.2_01) to give a working AOEL of 0.059 mg/kg.

Percentage AOEL values can then be calculated for each of the exposure scenarios and are summarised in the table below.

Exposure scenario	Systemic dose (mg/kg bw/d)		AOEL	% AOEL	
	95^{th} percentile	50^{th} percentile		95^{th} percentile	50^{th} percentile
Vac-pressure	0.0000069	0.000012	0.059	0.012	0.020
Dipping	0.000092	0.000017	0.059	0.156	0.029
Spraying cabinets	0 to 0.1 ppm (EASE)		0.059	<<100%	

Taking into account the use of PPE and industrial practises, very low vapour pressure and non-volatile nature of the active substance, exposure via the inhalation route is expected to be limited. In the interest of minimising unnecessary animal testing, further repeated dose/subchronic studies via the inhalation route are therefore not considered necessary.

Section 6.3.3, 6.4.3 **Annex Point IIA 6.3, 6.4**	**Repeated Dose / Subchronic Toxicity (Inhalation)**
	It is also important to note that potential inhalation toxicity is secondary to the potential local (irritant) effects as cypermethrin is classified as a respiratory irritant (R37). This would also be a problem in terms of conducting further subchronic studies in animals.
Undertaking of intended data submission [ ]	Not applicable
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April, 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 A6.4.1 (01) Repeated dose toxicity

Annex Point IIA 6.4.1

Subchronic Oral Toxicity - Rat

			Official use only
		1 REFERENCE	
1.1	**Reference**	Hend, R.W., Butterworth S.T.G. (1976);Toxicity studies on the insecticide WL 43467 (cypermethrin): three month feeding study in rats; Shell UK Ltd., report no. TLGR.0027.76 (CYP/T3), May 1976 (unpublished).	
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**	No Existing study partly in compliance with method B.26 of Directive 87/302/EEC and OECD guideline 408 (1981)	
2.2	**GLP**	No GLP was not compulsory at the time the study was performed	
2.3	**Deviations**	Yes Histopathology not performed on all organs. Target organs not examined at all dose levels.	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	WL 43467 (cypermethrin)	
3.1.1	Lot/Batch number	21	
3.1.2	Specification	Deviating from specification given in section 2 as follows	
3.1.2.1	Description	liquid	
3.1.2.2	Purity	98.5%	
3.1.2.3	Stability	Stable	X
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	CD (SPF)	
3.2.3	Source	Charles River Ltd., Kent	
3.2.4	Sex	Males and females	
3.2.5	Age/weight at study initiation	5 weeks	
3.2.6	Number of animals per group	12 female rats per treatment group (11 for 25 ppm group) 24 males and 23 females in each control group respectively	X
3.2.7	Control animals	Yes	

Section A6.4.1 (01) Repeated dose toxicity

Annex Point IIA 6.4.1 **Subchronic Oral Toxicity - Rat**

3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	90 days
3.3.2	Frequency of exposure	Daily
3.3.3	Postexposure period	None
3.3.4	**Oral**	
3.3.4.1	Type	In food
3.3.4.2	Concentration	0, 1.25, 5, 20, and 80 mg/kg /d ad libitum (0, 25, 100, 400 and 1600 ppm)
3.3.4.3	Vehicle	Not mentioned in report
3.3.4.4	Concentration in vehicle	Not mentioned in report
3.3.4.5	Total volume applied	Not mentioned in report
3.3.4.6	Controls	vehicle, plain diet or other
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes (daily observation)
3.4.1.2	Mortality	Yes (recorded daily)
3.4.2	Body weight	Yes (recorded weekly)
3.4.3	Food consumption	Yes (recorded weekly)
3.4.4	Water consumption	No
3.4.5	Ophthalmoscopic examination	No
3.4.6	Haematology	Yes (at necropsy)
3.4.7	Clinical Chemisty	Yes (at necropsy)
3.4.8	Urinalysis	No
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	Yes organs: liver, kidneys, testes, spleen, brain, heart
3.5.2	Gross and histopathology	Yes Control, 400 and 1600 ppm groups organs: brain, heart, kidney, lung, spleen, liver, alimentary tract, pancreas, salivary gland, thymus, mesenteric lymph node, gonads, prostate or uterus, pituatry, adrenals, larynx, thyroid, eye and sciatic nerve.

Section A6.4.1 (01) **Repeated dose toxicity**

Annex Point IIA 6.4.1 **Subchronic Oral Toxicity - Rat**

3.5.3	Other examinations	None
3.5.4	Statistics	Yes, Body and organ weights analysed by covariance. Chemical and haematological parameters examined using analysis of variance.
3.6	**Further remarks**	-
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	In the 1600 ppm group, many of the animals showed hypersensitivity and abnormal gait during the first 5 weeks of the experiment. Clinical recovery was observed after the end of the 5th week and their food intake increased to a normal level. The general health and behaviour of animals in dose groups up to 400 ppm were unaffected by ingestion of cypermethrin.
4.1.2	Mortality	In the 1600 ppm group , 1 male died and 3 were killed for humane reasons.
4.2	**Body weight gain**	See table A6.4.1_01_1
4.3	**Food consumption and compound intake**	See table A6.4.1_01_1
4.4	**Ophtalmoscopic examination**	Not performed
4.5	**Blood analysis**	
4.5.1	Haematology	See table A6.4.1_01_1
4.5.2	Clinical chemistry	See table A6.4.1_01_1
4.5.3	Urinalysis	Not performed
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	See table A6.4.1_01_1
4.6.2	Gross and histopathology	Two of the male rats in the 1600 ppm group showed axon breaks and vacuolation of myelin in the sciatic nerve.
4.7	**Other**	The MTD was reached
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	12 CD (SPF) rats/sex were fed a diet containing cypermethrin, WL 43467 (B.n°.21, 98.5 %) at concentrations of 0, 25, 100, 400 and 1600 ppm over a period of 13 weeks (converted dose: 1.25, 5, 20 and 80 mg/kg/d).

Section A6.4.1 (01)	**Repeated dose toxicity**	
Annex Point IIA 6.4.1	**Subchronic Oral Toxicity - Rat**	
5.2	**Results and discussion**	The MTD was reached. In the 1600 ppm group many of the animals showed hypersensitivity and abnormal gait during the first 5 weeks of the experiment. Of these group, 1 male died and 3 were killed for humane reasons. Two of these rats showed axon breaks and vacuolation of myelin in the sciatic nerve. Clinical recovery was observed after the end of the 5th week and their food intake increased to a normal level. The general health and behaviour of animals in dose groups up to 400 ppm were unaffected by ingestion of cypermethrin. .
5.3	**Conclusion**	
5.3.1	LO(A)EL	
5.3.2	NO(A)EL	100 ppm = 5 mg/kg bw, later updated to 20 mg/kg bw (see point 5.3.5 below)
5.3.3	Other	
5.3.4	Reliability	2
5.3.5	Deficiencies	Yes. Protocol not fully in compliance with test method B of Directive 87/302/EEC or OECD guideline 408 (1981). However the study is considered acceptable as it was carried out at an established facility and using cypermethrin of known purity. Study evaluated and accepted under Directive 91/414/EC. The NOAEL of 5 mg/kg was assigned in the original monograph. However during the ECCO review process this was subsequently changed to 20 mg/kg bw.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April, 2007.
Materials and Methods	The applicant's version is accepted with the following amendments: *2.3 Deviations:* No examination of water consumption, urinalysis, ophtalmoscopic examination. *3.1.2.3 Stability:* The stability of the test substance in the diet was not confirmed. *3.2.6 Number of animals per group:* 12 male and female rats per treatment group (except 11 females for the 25ppm group)
Results and discussion	The applicant's version is accepted.
Conclusion	The applicant's version is accepted: NO(A)EL: 20 mg/kg bw (400 ppm)
Reliability	2
Acceptability	acceptable

Section A6.4.1 (01) Repeated dose toxicity

Annex Point IIA 6.4.1

Subchronic Oral Toxicity - Rat

Remarks	
	COMMENTS FROM ... *(specify)*
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_4_1_01_1: Rat-90 day feeding study – Results

Endpoinst/dose	0		25 ppm		100 ppm		400 ppm		1600 ppm	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Mortality									3	
Clinical signs									ataxia, spayed hind limbs hypersensitivity	
Body weight									↘ 17%	↘ 10%
Food intake									↘	↘
Hematology										
Hb									↘ 4%	↘ 6%
PCV										↘ 7%
RBC										↘ 6%
KCCT									↘ 11%	
Clinical chemistry										
Proteins										↘ 6%
K+									↗ 13%	
AP										↗ 40%
Urea									↗ 20%	↗ 39%
Mean Organ weight (adjusted for terminal bodyweight)										
Liver										↗ 10%
Kidney							↗ 5%		↗ 7%	↗ 14%
Spleen										↗ 17%

Section A6.4.1 (02) Repeated dose toxicity

Annex Point IIA 6.4.1 **Subchronic Oral Toxicity - Dog**

			Official use only
		1 REFERENCE	
1.1	**Reference**	Buckwell, A., Butterworth, S. (1977); A 13 week feeding study of WL 43467 (cypermethrin) in dogs; Shell UK Ltd., report no. TLGR.0127.77 (CYP/T9), November 1977 (unpublished).	
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**	No	
		Existing study comparable to method B of Directive 87/302/EEC (corresponding OECD guideline 408)	X
2.2	**GLP**	No	
		GLP was not compulsory at the time the study was performed	
2.3	**Deviations**	Yes	
		Means: standard deviations not given	
		3 MATERIALS AND METHODS	
3.1	**Test material**	WL 43467 (cypermethrin)	
3.1.1	Lot/Batch number	30	
3.1.2	Specification	Deviating from specification given in section 2 as follows	
3.1.2.1	Description	liquid	
3.1.2.2	Purity	98.%	
3.1.2.3	Stability	Stable	X
3.2	**Test Animals**		
3.2.1	Species	Dog	
3.2.2	Strain	Beagle	
3.2.3	Source	Accredited breeder	
3.2.4	Sex	Males and females	
3.2.5	Age/weight at study initiation	8 – 11 months	
3.2.6	Number of animals per group	4 male, 4 female	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral	

Section A6.4.1 (02) Repeated dose toxicity

Annex Point IIA 6.4.1 **Subchronic Oral Toxicity - Dog**

3.3.1	Duration of treatment	90 days
3.3.2	Frequency of exposure	Daily
3.3.3	Postexposure period	None
3.3.4	**Oral**	
3.3.4.1	Type	In food
3.3.4.2	Concentration	0, 0.125, 1.25, 12.5, 37.5 mg/kg /d ad libitum (0, 5, 50, 500, 1500 ppm)
3.3.4.3	Vehicle	A.R. grade acetone
3.3.4.4	Concentration in vehicle	Not mentioned in report
3.3.4.5	Total volume applied	400g daily of powdered diet moistened with 400ml water
3.3.4.6	Controls	Powdered diet plus equivalent volume of vehicle
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes (daily observation)
3.4.1.2	Mortality	Yes (recorded daily)
3.4.2	Body weight	Yes (recorded weekly)
3.4.3	Food consumption	Yes (daily observation)
3.4.4	Water consumption	No
3.4.5	Ophthalmoscopic examination	Yes (at start and end of the study)
3.4.6	Haematology	Yes (weeks 0, 1, 4, 8 and 13)
3.4.7	Clinical Chemisty	Yes (weeks 0, 1, 4, 8 and 13)
3.4.8	Urinalysis	No
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	Yes organs: liver, kidneys, testes, adrenal, brain, heart, thyroid
3.5.2	Gross and histopathology	Yes All dose groups organs: brain, heart, liver, aorta, spleen, kidneys, gonads, stomach, pancreas, lymph nodes, prostate/uterus, thyroid, thymus, eye, lungs, pituatry, adrenals, intestine, sciatic nerve, posterior tibial nerve, salivary glands, urinary bladder, gall bladder.
3.5.3	Other examinations	None

Section A6.4.1 (02)		**Repeated dose toxicity**	
Annex Point IIA 6.4.1		**Subchronic Oral Toxicity - Dog**	
3.5.4	Statistics	Yes, Body and organ weights analysed by covariance. Chemical and haematological parameters examined using analysis of variance.	
3.6	**Further remarks**	-	
		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	Feeding 1500 ppm caused diarrhoea, licking and chewing of the paws, whole body tremors, a stiff exagegerated hind leg gait, ataxia, incoordination and hyperaesthesia in all except one female animal (which refused to eat the whole of her daily ration) in the dose group.	
4.1.2	Mortality	In the high dose group, 2 males and 2 females were sacrificed during week 6 and 10, 10 and 12 for humane reasons.	
4.2	**Body weight gain**	loss of 17-18% in the top dose group. This is though to be due to the inappetence oberved in intoxicated dogs at the 1500 ppm dose level.	X
4.3	**Food consumption and compound intake**	Reduced food intake in the top dose group.	X
4.4	**Ophtalmoscopic examination**	No compound related effects.	
4.5	**Blood analysis**		
4.5.1	Haematology	RBC count (↘ 6%) and and KCCT (↘ 21%) were significantly reduced in females at the top dose . Other minor differences were not attributable to the compound.	
4.5.2	Clinical chemistry	Minor difference not attributable to the test compound.	
4.5.3	Urinalysis	Minor difference not attributable to the test compound.	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	No effects on organ weights which could be attributed to the test compound up to the 500 ppm dose level.	
4.6.2	Gross and histopathology	In the top dose group, animals showed non-specific pathological changes, mainly in the lungs, where focal bronchopneumonia was seen in several animals. Such changes can be expected in severe intoxication from any cause.	
4.7	**Other**		
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	4 Beagle dogs/sex/dose were fed a diet containing cypermethrin, WL43467(cypermethrin) (B.n°.30; 98%) at concentrations of 0, 5, 50, 500 or 1500 ppm over a period of 13 weeks. Converted dose : 0, 0.125, 1.25, 12.5, 37.5 mg/kg/d.	
5.2	**Results and discussion**	Clinical signs, were observed in the high dose group only along with reduced food consumption and bodyweight. No significant histopathological changes were attributed to the test compound.	

Section A6.4.1 (02) **Repeated dose toxicity**

Annex Point IIA 6.4.1 **Subchronic Oral Toxicity - Dog**

5.3	**Conclusion**	
5.3.1	LO(A)EL	
5.3.2	NO(A)EL	500 ppm = 12.5 mg/kg bw
5.3.3	Other	
5.3.4	Reliability	2
5.3.5	Deficiencies	Yes. Protocol not fully in compliance with the method B of Directive 87/302/EEC or OECD 408(1981). Minor deviations from the official protocol. However the study is considered acceptable as it was conducted at an established research facility and used cypermethrin of known purity 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	April, 2007.
Materials and Methods	The applicant's version is acceptable with the following amendments: *2.1 Guideline:* The study is partly in compliance with method B.27 of Directive 87/302/EEC and OECD guideline 409 (1981). *3.1.2.3 Stability:* The stability of the test substance in the diet was not confirmed.
Results and discussion	The applicant's version is adopted with the following amendments: *4.2 Body weight gain and 4.3 Food consumption and compound intake:* The BE CA cannot verify the results because the data (table I) were not included in the report made available. *4.5.1 Haematology:* RBC count (↘ 6%) and and KCCT (↘ 15%) were significantly reduced in females at the 500 ppm dose at the end of the study (13 weeks). Other minor differences were not attributable to the compound. However, the KCCT values of the female dogs fed 500 ppm were consistently lower throughout the study (also at the start of the study) compared with the controls. No significant differences were found for male animals.
Conclusion	NO(A)EL: 12.5 mg/kg bw (500 ppm) The applicant's version is adopted.
Reliability	3 (2 when requested data is provided)
Acceptability	Will be acceptable when the body weight and food intake data will be provided.
Remarks	The data concerning body weight and food intake (nor raw data, nor mean ±SD) were not made available to the BE CA. These data were not included in the report made available.

Section A6.4.1 (02) Repeated dose toxicity

Annex Point IIA 6.4.1

Subchronic Oral Toxicity - Dog

	COMMENTS FROM ... *(specify)*
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 A6.5, A6.7 Chronic Toxicity / Carcinogenicity

Annex Point IIA6.5, IIA6.7 **Rat-combined chronic toxicity/carcinogenicity study**

			Official use only
		1 REFERENCE	
1.1	**Reference**	McAusland, H., Butterworth, S., Hunt, P.F. (1978); Toxicity studies on the insecticide WL 43467 (cypermethrin): A 2 year feeding study in rats; Shell International Chemical Company, report no. TLGR.78.189 (CYP/T10), December 1978 (unpublished).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Chimac-Agriphar s.a.	
1.2.2	Companies with letter of access		
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**	No	
		Existing study. Method used comparable to method B.30 of Directive 87/302/EEC (corresponding OECD guideline 453)	
2.2	**GLP**	No	
		GLP was not compulsory at the time the study was performed	
2.3	**Deviations**	Yes	
		Low number of rats. Blood albumin, glucose, GGT and ornithine decarboxylase were not measured. Urinalysis was not performed.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	WL 43467 (cypermethrin), cis:trans ratio:1/1	
3.1.1	Lot/Batch number	30	
3.1.2	Specification	Deviating from specification given in section 2 as follows	
3.1.2.1	Description	Liquid	
3.1.2.2	Purity	98%	
3.1.2.3	Stability	Not specified in report	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Wistar	
3.2.3	Source	Shell Toxicology Laboratory	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	5 weeks	
3.2.6	Number of animals per group	24 animals/sex/dose	

Section A6.5, A6.7		**Chronic Toxicity / Carcinogenicity**
Annex Point IIA6.5, IIA6.7		**Rat-combined chronic toxicity/carcinogenicity study**
3.2.6.1	at interim sacrifice	16 animals/sex/dose sacrificed at 6 or 12 months
		12 animals/sex/dose sacrificed at 18 months
3.2.6.2	at terminal sacrifice	24 animals/sex/dose sacrificed after 2 year exposure period
3.2.7	Control animals	Yes (48 animals/sex)
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	24 months
3.3.2	Interim sacrifice(s)	After 6, 12 and 18 months
3.3.3	Final sacrifice	After 24 months
3.3.4	Freqency of exposure	Daily
3.3.5	Postexposure period	None
		Oral
3.3.6	Type	In food
3.3.7	Concentration	0, 1, 10, 100, 1000 ppm (0, 0.05, 0.5, 5, 50 mg/kg bw) food consumption ad libitum
3.3.8	Vehicle	For 1000ppm diet: dissolved in a minimal amount (100ml) of A.R. grade acetone
		For 100, 10, 1 ppm diets: further dilution of 1000ppm diet with LAD 2. diet
3.3.9	Concentration in vehicle	See above
3.3.10	Total volume applied	See above
3.3.11	Controls	Vehicle plus LAD 2 diet
3.4	**Examinations**	
3.4.1	Body weight	Yes (weekly for the first 13 weeks, thereafter at four weekly intervals)
3.4.2	Food consumption	Yes (weekly for the first 13 weeks, thereafter at four weekly intervals)
3.4.3	Water consumption	No
3.4.4	Clinical signs	Yes
3.4.5	Macroscopic investigations	Tumour incidence and classification
3.4.6	Ophthalmoscopic examination	No
3.4.7	Haematology	Yes

Section A6.5, A6.7 **Chronic Toxicity / Carcinogenicity**

Annex Point IIA6.5, IIA6.7 **Rat-combined chronic toxicity/carcinogenicity study**

		Number of animals:	all animals
		Time points:	End of study/necropsy (6, 12 18 and 24 months)
		Parameters:	Total red and white cell counts, haemoglobin concentration and mean cell volumes.
			Differential white cell counts performed on top dose, and control group plus other dose levels if haematological abnormalities observed. Also on the 100ppm group at the 6 moth necropsy.
			Citrated blood used to estimate prothrombin and kaolin-cephalin coagulation times.
3.4.8	Clinical Chemistry	Yes	
		Number of animals:	All animals
		Time points:	End of study/necropsy (6, 12 18 and 24 months)
		Parameters:	Total plasma protein, urea and chloride levels, alkaline phosphatase activity, AST/ALT activity, plasma sodium/potassium levels.
3.4.9	Urinalysis	No	
3.4.10	Pathology	Yes	
3.4.10.1	Organ Weights	Yes	
		From:	all surviving animals
		Organs:	Liver, kidneys, testes, spleen, brain, heart
3.4.11	Histopathology	Yes	
		from:	All animals in the control and two highest dose groups (including those dying early)
			All animals in the lower dose groups in the 2 year group
		Organs:	Brain, pituitary, thyroid, parathyroid, thymus, oesophagus, salivary glands, stomach, small and large intestines, liver, pancreas, kidneys, adrenals, spleen, heart, trachea, lungs, gonads, prostate or uterus, urinary bladder, mesenteric lymph nodes, sciatic nerve, eye and lachrimal glands
			Tissues stored for reference: Knee joint and femur, femoral muscle, mammary gland, seminal vesicles, spinal cord, tongue, bone marrow smear.
3.4.12	Other examinations	None	

Section A6.5, A6.7 **Chronic Toxicity / Carcinogenicity**

Annex Point IIA6.5, IIA6.7 **Rat-combined chronic toxicity/carcinogenicity study**

3.5	**Statistics**	Body and organ weights were analysed by covariance analysis using initial bodyweight or terminal organ weight as the covariate respectively. Reported means were adjusted for covariance, where no significant covariance relationship was found unadjusted means were reported. Clinical chemical and haematological parameters were examined using analysis of variance. Any significant differences between the treated and control group means was tested using the Williams t-test. On occasions where a monotonic dose response could not be assumed, the Dunnett's test was used.
3.6	**Further remarks**	-
		4 RESULTS AND DISCUSSION
4.1	**Body weight**	Reduction in bodyweight seen in top dose 2 year males and females. For top dose animals in the interim groups, the reduction was statistically significant from weeks 1 to 32 (males), and 1 to 46 and week 76 (females)
4.2	**Food consumption**	Small reductions (not always statistically significant) in food consumption were seen during the first 13 weeks in the 1000 ppm group (both 2 year and ad interim animals).
4.3	**Water consumption**	Not determined
4.4	**Clinical signs**	No effects considered to be of toxicological significance
4.5	**Macroscopic investigations**	No effects considered to be of toxicological significance
4.6	**Ophthalmoscopic examination**	Not determined
4.7	**Haematology**	Minor fluctuations were seen in various parameters in the interim and 2 year groups but these were not considered to be of toxicological significance as they were minor in nature with no evidence of compound related tissue damage.
4.8	**Clinical Chemistry**	No effects considered to be of toxicological significance
4.9	**Urinalysis**	Not determined
4.10	**Pathology**	No compound related gross pathological changes in any tissues
4.11	**Organ Weights**	See table A6 5-1
4.12	**Histopathology**	Sciatic nerves from many animals at 1 year and later showed very small numbers of nerve fibres exhibiting the changes of Wallerian degeneration. The incidences increased with age but there was no difference in severity between the dose groups.
4.13	**Other examinations**	None
4.14	**Time to tumours**	See table A6 5-2. Statistical analysis showed no evidence of increased risk of tumour development over the 2 year period.

Section A6.5, A6.7 **Chronic Toxicity / Carcinogenicity**

Annex Point IIA6.5, IIA6.7 **Rat-combined chronic toxicity/carcinogenicity study**

4.15	**Other**	The most common tumour was pituitary adenoma, which occurred in 39 of 48 female controls in the 2 year group and was the cause of early death or removal from the trial in 19 of these. Similar incidences were found in other dose levels in females. These were present, but much less common, in males and were believed not to be treatment-related. A cluster of 4 uterine tumours in the 100 ppm was reported. Since there were no uterine tumours at 1000 ppm and there was no question of competing toxicity at the higher level preventing their development. These uterine tumours at 100 ppm were not considered to be compound related. Statistical analysis revealed no evidence of increased risk of tumour development following dietary inclusion of cypermethrin for up to 2 years.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	24 Wistar rats/sex/dose received in the diet WL43467(cypermethrin) (B.n°.30, 98%; cis/trans ratio : 1/1) at 0, 1, 10, 100, 1000 ppm for approximately 24 months. 48 animals/sex were used in the control group. Additional groups of 6 rats/sex/dose were sacrificed after 6 or 12 months and 12 rats/sex/dose were sacrificed after 18 months. Only 24 rats/sex/dose for the 2 year exposure period. Mean cypermethrin content of diets fed to rats over a 2 year period: 1.04, 10.0, 99.0 and 1002 ppm. Converted dose: : 0, 0.05, 0.5, 5 and 50 mg/kg/d .
5.2	**Results and discussion**	Minor fluctuations were seen in various haematological parameters in the interim and 2 year groups but these were not considered to be of toxicological significance.
5.3	**Conclusion**	Cypermethrin is not carcinogenic in this study NOAEL = 100 ppm = 5 mg/kg bw/day
5.3.1	Reliability	2
5.3.2	Deficiencies	Yes. Low number of rats ; blood albumin, glucose, GGT and ornithine decarboxylase were not measured. Urinalysis was not performed. However the study is considered acceptable as it was conducted at an established facility and used cypermethrin of known purity. Study evaluated and accepted under Directive 91/414/EC.

Section A6.5, A6.7 Chronic Toxicity / Carcinogenicity

Annex Point IIA6.5, IIA6.7 **Rat-combined chronic toxicity/carcinogenicity study**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April, 2007.
Materials and Methods	The applicant's version is acceptable.
Results and discussion	The applicant's version is adopted.
Conclusion	NOAEL = 100 ppm = 5 mg/kg bw/d The applicant's version is adopted.
Reliability	2
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_5-1. Table for 2 year toxicity study of cypermethrin in rats

Endpoint/dose	**0**		**1 ppm**		**10 ppm**		**100 ppm**		**1000 ppm**	
Survival rate (%)	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
6 months	100	100	100	100	100	100	100	100	100	100
12 months	100	83	100	100	100	83	100	100	100	100
18 months	100	71	92	83	75	67	100	83	100	100
24 months	67	42	46	33	54	38	71	42	71	50
Clinical signs	sore hocks , gross external masses, mammary tumors, general poor conditions and loss of appetite, not compound related									
Food consumption									(↘ 7%)	(↘ 10%)
Body weight									↘ 7%	↘ 7% w 1-75
Hematology: PT										↗ 4%
Clinical chemistry:										
liver PNOD									↗	↗
urea									↗ 58% 2 y	
AP					↘ 16% 2 y		↘ 18% 2 y		↘ 33% 2 y	
Na^+									↗ 1%	
Rel. organ weight										
testes									abs↘ 6 mth	
liver									abs+rel ↗ 18 mth	
heart									rel↗ 6 mth, abs↘ 12 mth	
kidney							abs↗ 18 mth		rel↗ 12mth, abs↗ 18 mth	rel↗ 6 mth
Histopathology: sciatic nerve degeneration : number affected/number survivors										
at 12 mth	2/12	1/12					0/6	2/6	1/6	2/6
at 18 mth	9/24	2/17					4/12	2/10	5/12	0/12
at 24 mth	17/31	10/20	8/11	3/8	11/13	4/9	10/17	5/9	12/17	5/12
Total	28/67	13/49					14/35	9/24	18/35	7/30

%	42	26.5					40	37.5	51.4	23

Statistically significant ↗ or ↘ : covariance analysis; variance analysis; Williams t test or Dunnett's test

() not statistically significant

Table A6_5-2 Table for tumour incidence

Tissue/pathological finding % affected animals	**0**		**1 ppm**		**10 ppm**		**100 ppm**		**1000 ppm**	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Pituitary										
anterior lobe adenoma (TA) (B)	33	79	20	91	29	100	20	79	25	75
anterior lobe carcinoma (M)		6.2	4.1		4.1		4.1		4.1	
intermediate lobe adenoma (B)					4.1					
posterior lobe-asrrocytoma (M)	2.08								4.1	
Mammary glands										
fibroadenoma (B)		14				33		16		
adenocarcinoma (M)		28		63		33		16		40
Uterus										
adenocarcinoma (M)				4.1				4.1		
endometrial sarcoma (M)								8		

(B) benign; (M) malignant

Section A6.5 **Annex Point IIA.VI.6.5**	**Chronic toxicity test – Non-rodent species**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [√]	**Technically not feasible [ ]** **Scientifically unjustified [√]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	From the results of the chronic toxicity study in the rat and comparing this against the 90-day subchronic toxicity study, there is no apparent change in the actual No Observed Adverse Effect Levels (see Doc IIIA6.4.1_01 and Doc IIIA6.5/6.7). This suggests that there is no increase in the overall toxicity of the material following prolonged dosing. It is seen, from the Absorption, Distribution and Excretion study in the rat (Doc IIIA6.2_01) that repeated exposure to the test material over 10 days does cause an increase in blood and tissue concentrations (particularly inguinal and perirenal fat). This increase in concentrations does not alter the overall toxicity, when exposure continues over prolonged periods. Clearance is also fairly rapid following cessation of treatment. The results of the subchronic repeat dose study in the dog (Doc IIIA6.4.1_02) also shows similar target organ toxicity to that observed in the rat. There was no significant reduction in the No Observed Adverse Effect Level for the dog subchronic study when compared to the equivalent rat study. For these reasons, and to minimise any unnecessary animal testing, it is concluded that sufficient data has been collected from the existing studies to enable a suitable prediction of the adverse effects of chronic exposure to the test material.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	April, 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*	

Section A6.5 **Annex Point IIA.VI.6.5**	**Chronic toxicity test – Non-rodent species**
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.6.2 Genotoxicity in vitro

Annex Point IIA 6.6.2 **In-vitro cytogenetic study in mammalian cells**

			Official use only
		1 REFERENCE	
1.1	**Reference**	Oláh , B. (2002); In vitro mammalian chromosomal aberration study of Cypermethrin cis:trans/40:60; Toxicology Research Centre Ltd, report no. 01/569-020C (CYP/T320), 6 August 2002 (unpublished) Dates of experimental work: 10 July 2001 – 25 July 2001	
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, OECD guideline 473 (1997)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Cypermethrin cis:trans/40:60	
3.1.1	Lot/Batch number	2001044557	
3.1.2	Specification	Cis:trans = 40.2/59.8	
3.1.2.1	Description	Pale yellow viscous liquid	
3.1.2.2	Purity	93.6% w/w	
3.1.2.3	Stability	Stability not given	
3.2	**Study Type**	In Vitro mammalian chromosome aberration test	
3.2.1	Organism/cell type	Chinese hamster Ovary (CHO), subline KI	
3.2.2	Deficiencies / Proficiencies	Not applicable	
3.2.3	Metabolic activation system	S9 mix Male Wistar rats were treated orally by gavage with Phenobarbitone (PB) and β-naphtoflavone (BNF) (80 mg/kg respectively) on days 3,2 and 1 before sacrifice on day 5. Livers were removed aseptically, washed in 0.15M KCl and homogenised. Homogenates were centrifuged and the supernatant decanted and retained as the S9 fraction.	
3.2.4	Positive control	Ethyl methanesulphonate and N-Nitrosodimethylamine	
3.3	**Administration / Exposure; Application of test substance**		
3.3.1	Concentrations	10, 50 and 100 µg/ml Doses were selected on the basis of a preliminary cytotoxicity assay using concentrations of 1, 10, 50, 100, 200, 400, 800 and 1600 µg/ml cypermethrin in the presence and absence of S9 mix.	

Section A6.6.2		**Genotoxicity in vitro**	
Annex Point IIA 6.6.2		**In-vitro cytogenetic study in mammalian cells**	
3.3.2	Way of application	The study was conducted as two separate experiments Study 1 The test substance cypermethrin was dissolved in dimethylsulphoxide (DMSO) (up to 10 mg/ml). Duplicate cultures were used for each test concentration and the vehicle control. $2x10^6$ seeded cells (10^6 cells/dish) were treated in each group. The exposure period was 4 hours at 37°C, after which the cells were washed twice with F12 medium. The growth medium was then added. Samples were taken at approximately 1.5 normal cell cycle from the beginning of treatment. Study 2 As above, however the exposure period was 1.5 normal cell cycle length. After the exposure period, cells were washed twice with F12 medium and then sampled.	
3.3.3	Pre-incubation time	The Exposure period was 4 hours at 37°C (study 1) or 1.5 normal cell cycle length = 18-20 hours (study 2). Cell cultures were treated with 0.5 µg/ml colchicines for 2 hours prior to harvesting. Cells were then swollen with 0.075M KCl and washed three times in fixative (3: 1/methanol:acetic acid). Once the preparation was free of plasma, cells were placed on slides and air dried.	
3.4	**Examinations**		
3.4.1	Number of cells evaluated	Slide preparations were Giemsa stained and all slides were scored blind. 200 metaphase cells containing 2N±2 centromeres were evaluated for structural aberrations in each experimental group. Chromatid and chromosome aberrations were recorded separately (including gaps, deletions and exchanges).	
3.5	**Statistics**	CHI^2 test was used for statistical analysis.	
		4 RESULTS AND DISCUSSION	
4.1	**Genotoxicity**	See tables A6_6_2-1 and A6_6_2-2	X
4.1.1	without metabolic activation	No significant increase in the number of aberrations without gaps	
4.1.2	with metabolic activation	No significant increase in the number of aberrations without gaps	
4.2	**Controls**	Both positive controls (ethyl methanesulphonate and N-nitrosodimethylamine) showed a clear clastogenic effect, validating the test. In the untreated control, the number of aberrations without gaps was <5%, demonstrating suitability of the cell line used in the study.	

Section A6.6.2 **Genotoxicity in vitro**

Annex Point IIA 6.6.2 **In-vitro cytogenetic study in mammalian cells**

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Test item was studied for clastogenic activity in CHO cells both in the presence and absence of metabolic activation. Two independent experiments were performed with an exposure period of 4 hours and 1.5 normal cell cycle length respectively (18-20 hours).
5.2	**Results and discussion**	There was no significant increase in the number of aberrations without gaps in either the presence or absence of metabolic activation in either study. The positive controls showed a clear clastogenic effect thereby validating the test.
5.3	**Conclusion**	Cypermethrin cis:trans/40:60 proved to be non-clastogenic
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, 2007.
Materials and Methods	The applicant's version is acceptable.
Results and discussion	The applicant's version is adopted with the following amendments: The tables A6_6_2-1 and A6_6_2-2 are adapted and table A6_6_2-3 is added.
Conclusion	Cypermethrin cis:trans/40:60 does not induce chromosome aberrations, under the test conditions used, in cultured CHO-cells.
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_6_2-1. Chromosome Aberration Assay: Experiment 1, exposure time = 4 hours at 37°C

		Untreated control		Vehicle control		Pos Control*		low dose 10µg/ml		mid dose 50µg/m		high dose 100µg/m	
		a	b	a	b	a	b	a	b	a	b	a	b
Without S9													
chromosome aberrations	gap	1	1	1	2	7	4	2	2	1	4	5	2
	del	0	0	0	0	4	7	1	0	0	0	1	1
	exchange	1	1	0	1	2	1	0	0	1	0	0	0
chromatid aberrations	gap	2	3	6	3	17	22	5	7	8	4	5	4
	del	1	1	2	1	14	16	1	1	1	1	1	0
	exchange	0	0	0	0	1	11	0	0	0	0	1	1
No. cells with aberrations (gap+)		3	4	6	5	18	17	7	8	8	7	9	6
No. cells with aberrations (gap-)		2	2	2	2	5	7	2	1	2	1	2	1
Abberation rate (aberrations/100 cells)		0.02	0.02	0.02	0.02	0.21	0.35	0.02	0.01	0.02	0.01	0.03	0.02
With S9													
chromosome aberrations	gap	2	3	1	2	6	3	2	4	1	4	2	2
	del	0	0	1	0	8	5	0	0	1	0	0	0
	exchange	0	0	0	1	3	2	1	0	0	0	1	1
chromatid aberrations	gap	2	2	3	4	19	14	6	3	8	3	3	6
	del	1	1	1	1	17	14	0	1	0	2	3	1
	exchange	0	0	0	0	2	0	0	0	0	0	0	0
No. cells with aberrations (gap+)		4	5	4	6	17	14	7	7	7	6	5	8
No. cells with aberrations (gap-)		1	1	1	1	10	12	1	1	1	1	3	2
Abberation rate (aberrations/100 cells)		0.01	0.01	0.02	0.02	0.30	0.21	0.01	0.01	0.01	0.02	0.04	0.02

* N-Nitrosodimethylamine (0.4µl/ml)

Table A6_6_2-2. Chromosome Aberration Assay: Experiment 2, exposure time = 18-20 hours at 37°C

		Untreated control		Vehicle control		low dose 10µg/ml		mid dose 50µg/m		high dose 100µg/m	
		a	b	a	b	a	b	a	b	a	b
Without S9											
chromosome aberrations	gap	0	2	3	1	1	0	2	3	1	2
	del	0	0	0	0	1	1	0	0	1	0
	exchange	0	0	1	1	1	0	2	1	2	1
chromatid aberrations	gap	3	2	5	7	5	5	6	6	4	4
	del	1	1	1	1	2	1	0	0	1	2
	exchange	0	0	0	0	0	0	0	0	0	0
No. cells with aberrations (gap+)		4	4	6	8	5	5	8	9	5	6
No. cells with aberrations (gap-)		1	1	1	1	3	1	2	0	3	2
Abberation rate (aberrations/100 cells)		0.01	0.01	0.02	0.02	0.04	0.02	0.02	0.01	0.04	0.03
With S9											
chromosome aberrations	gap	1	2	1	1	2	1	2	2	1	2
	del	0	0	0	0	0	0	0	0	0	0
	exchange	0	0	0	0	1	0	1	0	0	0
chromatid aberrations	gap	4	2	6	5	2	4	3	3	8	4
	del	1	0	2	2	1	2	2	2	1	3
	exchange	0	0	0	0	0	0	0	2	1	0
No. cells with aberrations (gap+)		5	4	6	6	4	5	5	5	8	6
No. cells with aberrations (gap-)		1	04	2	2	1	2	3	3	1	2
Abberation rate (aberrations/100 cells)		0.01	0.00	0.02	0.02	0.02	0.02	0.03	0.04	0.02	0.03

Table A6_6_2-3. CHO Clonal toxicity data (treatment: 4h; cells seeded per dish: 200)					
Experiment 1					
Test condition	Dish 1	Dish 2	Dish 3	Average count ± SD	Relative survival (percent)*
Untreated control	204	206	209	206.33 ±2.52	10.81
Vehicle control (DMSO)	205	202	207	204.67 ± 2.52	100.00
Cypermethrin					
1 µg/ml	205	194	202	200.33 ± 5.69	97.88
10 µg/ml	181	192	198	190.33 ± 8.62	93.00
50 µg/ml	170	178	164	170.67 ± 7.02	83.39
100 µg/ml	92	103	98	97.67 ± 5.51	47.72
200 µg/ml	51	43	41	45 ± 5.29	21.99
400 µg/ml	0	0	0	0.00 ± 0.00	0.00
800 µg/ml	0	0	0	0.00 ± 0.00	0.00
1600 µg/ml	0	0	0	0.00 ± 0.00	0.00
Experiment 2					
Test condition	Dish 1	Dish 2	Dish 3	Average count ± SD	Relative survival (percent)
Untreated control	210	203	196	203.00 ± 7.00	100.83
Vehicle control (DMSO)	198	204	202	201.33 ± 3.06	100.00
Cypermethrin					
1 µg/ml	203	192	190	195.00 ± 7.00	96.41
10 µg/ml	180	173	181	178.00 ±4.36	88.41
50 µg/ml	162	161	170	164.33 ± 4.93	81.62
100 µg/ml	94	102	89	95.00 ± 6.56	47.19
200 µg/ml	19	21	22	20.67 ± 1.53	10.26
400 µg/ml	0	0	0	0.00 ± 0.00	0.00
800 µg/ml	0	0	0	0.00 ± 0.00	0.00
1600 µg/ml	0	0	0	0.00 ± 0.00	0.00

* Relative to vehicle control

Section A6.6.3 Genotoxicity in vitro

Annex Point IIA 6.6.3 **In-vitro gene mutation assay in mammalian cells**

			Official use only
		1 REFERENCE	
1.1	**Reference**	Flanders, L. (2011); Cypermethrin Technical – L5178Y TK +/- mouse lymphoma assay; Harlan Laboratories Ltd., report no. 41004533, 23 March 2011 (unpublished). Dates of experimental work: 30 November 2010 – 07 February 2011.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	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, OECD guideline 476	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		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	Pale yellow viscous liquid	
3.1.2.2	Purity	93.56% w/w	
3.1.2.3	Stability	Stable	
3.2	**Study Type**	In -vitro gene mutation study in mammalian cells	
3.2.1	Organism/cell type	Mouse lymphoma cell line L5178Y TK+/- 3.7.2c	
3.2.2	Deficiencies / Proficiencies	Not applicable	
3.2.3	Metabolic activation system	S9 mix (PB/βNF S9) prepared in-house from the livers of male Sprague-Dawley rats weighing approximately 250g. These had each received, orally, three consecutive daily doses of phenobarbital/β-naphthoflavone (80/100 mg per kg per day) prior to S9 preparation on the fourth day. S9-mix was prepared by mixing S9, NADP (5 mM), G-6-P (5 mM), KCl (33 mM) and $MgCl_2$ (8 mM) in R0. 20% S9-mix (i.e. 2% final concentration of S9) was added to the cultures of the Preliminary Toxicity Test and of Experiment 1. In Experiment 2, 10% S9-mix (i.e. 1% final concentration of S9), was added.	
3.2.4	Positive control	Ethylmethanesulphonate (EMS) in the absence of metabolic activation. Cyclophosphamide (CP) in the presence of metabolic activation.	

Section A6.6.3 Genotoxicity in vitro

Annex Point IIA 6.6.3 **In-vitro gene mutation assay in mammalian cells**

3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	Experiment 1 : 65 to 2080 µg/ml in both the absence and presence of metabolic activation. Experiment 2: 2.03 to 260 µg/ml in the absence of metabolic activation and 16.25 to 2080 µg/ml in the presence of metabolic activation.
3.3.2	Way of application	The study was conducted as two separate experiments Experiment 1 Several days before starting the experiment, an exponentially growing stock culture of cells was set up so as to provide an excess of cells on the morning of the experiment. The cells were counted and processed to give 1 x 10^6 cells/ml in 10 ml aliquots in R10 medium in sterile plastic universals. The treatments were performed in duplicate (A + B), both with and without metabolic activation (S9-mix) at six dose levels of the test item (65 to 2080 µg/ml in both the absence and presence of metabolic activation), vehicle and positive controls. To each universal was added 2 ml of S9-mix if required, 0.2 ml of the treatment dilutions, (0.2 ml for the positive control) and sufficient R0 medium to bring the total volume to 20 ml. Experiment 2 As in Experiment 1, an exponentially growing stock culture of cells was established. The cells were counted and processed to give 1 x 10^6 cells/ml in 10 ml duplicate cultures in R10 medium for the 4-hour treatment with metabolic activation cultures. In the absence of metabolic activation the exposure period was extended to 24 hours; therefore 0.3 x 10^6 cells/ml in 10 ml duplicate cultures were established in 25 cm^2 tissue culture flasks. To each culture 2 ml of S9-mix was added if required, 0.2 ml of the treatment dilutions, (0.2 ml for the positive control) and sufficient R0 medium to give a final volume of 20 ml (R10 is used for the 24-hour exposure group). The dose range of the test item was 2.03 to 260 µg/ml in the absence of metabolic activation, and 16.25 to 2080 µg/ml in the presence of metabolic activation.
3.3.3	Pre-incubation time	Experiment 1: The treatment vessels were incubated at 37°C for 4 hours. Experiment 2: The treatment vessels were incubated at 37°C with continuous shaking using an orbital shaker for 24 hours in the absence of metabolic activation and 4 hours in the presence of metabolic activation.
3.4	**Examinations**	
3.4.1	Number of cells evaluated	Cells were counted, diluted to 10^4 cells/ml and plated for mutant frequency (2000 cells/well) in selective medium containing in 96-well microtitre plates. Cells were also diluted to 10 cells/ml and plated (2 cells/well) for viability (%V) in non-selective medium. The daily cell counts were used to obtain a Relative Suspension Growth (%RSG) value.

Section A6.6.3 Genotoxicity in vitro

Annex Point IIA 6.6.3 **In-vitro gene mutation assay in mammalian cells**

3.5	**Statistics**	The experimental data was analysed using a dedicated computer program, Mutant 240C by York Electronic Research, which follows the statistical guidelines recommended by the UKEMS (Robinson W D et al, 1989).
		4 RESULTS AND DISCUSSION
4.1	**Genotoxicity**	See tables A6_6_3-1 and A6_6_3-2
4.1.1	without metabolic activation	No significant increase in mutant frequency at the TK +/- locus
4.1.2	with metabolic activation	No significant increase in mutant frequency at the TK +/- locus
4.2	**Controls**	Neither of the vehicle control mutant frequency values were outside the acceptable range of 50 to 200 x 10^{-6} viable cells. Both of the positive controls produced marked increases in the mutant frequency per viable cell.

Section A6.6.3 Genotoxicity in vitro

Annex Point IIA 6.6.3 **In-vitro gene mutation assay in mammalian cells**

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The study was conducted according to a method that was designed to assess the potential mutagenicity of the test item on the thymidine kinase, TK +/-, locus of the L5178Y mouse lymphoma cell line.

Two independent experiments were performed. In Experiment 1, L5178Y TK +/- 3.7.2c mouse lymphoma cells (heterozygous at the thymidine kinase locus) were treated with the test item at six dose levels, in duplicate, together with vehicle (solvent) and positive controls using 4-hour exposure groups both in the absence and presence of metabolic activation (2% S9). In Experiment 2, the cells were treated with the test item at eight dose levels using a 4-hour exposure group in the presence of metabolic activation (1% S9) and a 24-hour exposure group in the absence of metabolic activation.

The dose range of test item was selected following the results of a preliminary toxicity test and for the first experiment was 65 to 2080 µg/ml in both the absence and presence of metabolic activation. For the second experiment the dose range was 2.03 to 260 µg/ml in the absence of metabolic activation, and 16.25 to 2080 µg/ml in the presence of metabolic activation.

5.2 Results and discussion

The maximum dose level used for the 4-hour exposure groups in the mutagenicity test was limited by a combination of toxicity and the presence of precipitate effectively reducing exposure of the test item to the cells, the maximum dose level in the 24-hour exposure group was limited by toxicity. The vehicle (solvent) controls had acceptable mutant frequency values that were within the normal range for the L5178Y cell line at the TK +/- locus. The positive control items induced marked increases in the mutant frequency indicating the satisfactory performance of the test and of the activity of the metabolising system.

The test item did not induce any toxicologically significant dose-related increases in the mutant frequency at any dose level, either with or without metabolic activation, in either the first or the second experiment.

5.3 Conclusion Cypermethrin cis:trans/40:60 proved to be non-mutagenic

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	*Give date of action*
Materials and Methods	*State if the applicants version is acceptable or indicate relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers*
Conclusion	Other conclusions: *(Adopt applicant's version or include revised version)*
Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator*
Acceptability	acceptable / not acceptable *(give reasons if necessary, e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat is necessary.)*
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6_6_3.1 Summary of results – Experiment 1

Treatment (µg/ml)		4-Hours-S-9 %RSG	RTG	MF§	Treatment (µg/ml)		4-Hours+S-9 %RSG	RTG	MF§
0		100	1.00	114.02	0		100	1.00	96.50
65		78	0.96	88.92	65		74	0.62	129.29
130		72	0.77	108.02	130		70	0.67	128.11
260		69	0.80	102.37	260		72	0.62	127.23
520		68	0.74	87.78	520		66	0.50	123.24
1040	X	8	0.04	124.26	1040	X	7	0.02	204.89
2080	X	6	0.03	83.83	2080	X	6	0.02	177.29
Linear trend				NS	Linear trend				NS
EMS 400		70	0.49	867.47	CP 2		84	0.53	656.44

Table 6_6_3.2 Summary of results – Experiment 2

Treatment (µg/ml)		24-Hours-S-9 %RSG	RTG	MF§	Treatment (µg/ml)		4-Hours+S-9 %RSG	RTG	MF§
0		100	1.00	101.06	0		100	1.00	93.64
2.03	Ø	106			16.25		80	0.89	95.39
4.06		108	1.17	93.61	32.5		68	0.70	131.32
8.13		106	1.12	93.88	65		63	0.64	101.29
16.25		88	1.05	90.70	130		63	0.69	96.79
32.5		41	0.43	105.06	260		61	0.56	134.00
65		26	0.23	79.02	520		55	0.58	121.29
130		18	0.16	139.83	1040	X	6	0.04	153.81
260		14	0.13	104.12	2080	Ø	6		
Linear trend				NS	Linear trend				NS
EMS 150		75	0.51	958.36	CP 2		76	0.40	863.04

%RSG = Percentage Relative Suspension Growth

RTG = Relative Total Growth

MF = Mutation Frequency

Section A6.6.4 Genotoxicity in vivo

Annex Point IIA 6.6.4 **In-vivo bone marrow micronucleus study in mice**

Official use only

	1 REFERENCE	
1.1	**Reference**	Mohan, A. (2008); Genotoxicity evaluation of Cypermethrin technical by *in vivo* mouse micronucleus assay. IIBAT – International Institute of Biotechnology and Toxicology, Tamil Nadu, India. Report number 0805303 (unpublished) Dates of experimental work: 9 October 2008 – 15 November 2008
1.2	**Data protection**	Yes
1.2.1	Data owner	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 no. 474 (1997)
2.2	**GLP**	No information included in study report to indicate GLP compliance.
2.3	**Deviations**	No
	3 MATERIALS AND METHODS	
3.1	**Test material**	Cypermethrin technical
3.1.1	Lot/Batch number	CMN92T1193AR
3.1.2	Specification	Certificate of Analysis supplied with study report verified the batch was within accepted specification.
3.1.2.1	Description	Viscous yellowish liquid
3.1.2.2	Purity	93.5%
3.1.2.3	Stability	Stable – expiry date 19 June 2010
3.1.2.4	Maximum tolerable dose	50 mg/kg, determined in a range finding study.
3.2	**Test Animals**	
3.2.1	Species	Mouse
3.2.2	Strain	Swiss albino
3.2.3	Source	Bred in-house at IIBAT
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	6-8 weeks 23-27g (males and females)
3.2.6	Number of animals per group	5 males and 5 females per dose and sampling time. A total of 55 males and 55 females allocated to 11 treatment or control groups.
3.2.7	Control animals	Yes – vehicle/negative controls and positive control group
3.3	**Administration/ Exposure**	Oral in dose volume not exceeding 2 mL/100g bodyweight. Vehicle was corn oil
3.3.1	Number of applications	1 (one high dose group and the corresponding negative control received two doses on consecutive days)

Section A6.6.4 Genotoxicity in vivo

Annex Point IIA 6.6.4 **In-vivo bone marrow micronucleus study in mice**

3.3.2	Interval between applications	Not applicable except for one high dose group and the corresponding negative control that received two doses on consecutive days with 24 h interval.
3.3.3	Post-exposure period	24 and 48 h after treatment Positive control: 24 h after treatment; untreated control: 24 and 48 h after beginning of study and 24 hours after second treatment of one group. Low, intermediate and high dose groups – 24 and 48 h after initiation of treatment and for one high dose group, 24 h after second administration.
		Oral
3.3.4	Type	Gavage
3.3.5	Concentration	50 mg/kg (MTD), 25 mg/kg and 12.5 mg/kg
3.3.6	Vehicle	Corn oil
3.3.7	Concentration in vehicle	2.5, 1.25 and 0.625% w/v
3.3.8	Total volume applied	2 ml/10g bw
3.3.9	Controls	Corn oil (vehicle control) Cyclophosphamide 20 mg/kg (positive control)
3.4	**Examinations**	
3.4.1	Clinical signs	General health condition recorded daily, bodyweights recorded pre-treatment on Day 0.
3.4.2	Tissue	bone marrow
	Number of animals:	all animals
	Number of cells:	2000 PCE examined. The PCE:NCE ratio determined from 200 erythrocytes
	Time points:	24 or 48 h after treatment
	Type of cells	erythrocytes in bone marrow (PCE – immature or NCE - mature)
	Parameters:	Micronucleated PCEs polychromatic/normochromatic erythrocytes ratio
		4 RESULTS AND DISCUSSION
4.1	**Clinical signs**	No signs of toxicity are presented in the study report, even at the MTD. Some indications of observed toxicity are given for the range-finding investigation but these are not replicated in the main study.

Section A6.6.4 Genotoxicity in vivo

Annex Point IIA 6.6.4 **In-vivo bone marrow micronucleus study in mice**

4.2	**Haematology / Tissue examination**	Test substance did not induce significant increase in the number of micronucleated PCEs in either males or females at any dose level 24 or 48 hours after single treatment, or 24 hours after two administrations. The mean percentage of micronucleated polychromatic erythrocytes in the treated groups was consistently lower than the vehicle mean for males and females at the 24 h and 48 h assessment points in the low and intermediate groups and the high dose groups were similar to the controls. The ratio of PCE:NCE is theoretically anticipated to be close to unity, with higher ratios normally apparent in younger animals and a shift in the ratio indicates inhibition of cell division, a reduction in the PCE count and normally a reduction of the PCE:NCE ratio to less than 1. The PCE:NCE ratios for the negative controls were: 24 h males and females: 1.35 and 1.42 48 h males and females: 1.37 and 1.43 24 h after two treatments: 1.47 and 1.45 The PCE:NCE ratios for the positive controls were: 24 h males and females: 1.75 and 1.83 The PCE:NCE ratios for the low and intermediate dose treated group were: 24 or 48 h males and females: in range of 0.86 to 1.28 (indicating a slight reduction in the ration in comparison with controls) The PCE:NCE ratios for the high dose treated group were: 24 or 48 h males and females: in range of 0.69 to 0.85 after one or two administrations (indicating a significant reduction in the PCE:NCE ratio). See table A6_6_4-1 There were no marked differences in the frequencies of micronucleated polychromatic erythrocytes for either sex in any of the treatment groups.
4.3	**Genotoxicity**	Cypermethrin technical proved to be negative for mutagenicity in Swiss albino mice
4.4	**Other**	In the positive control group, cyclophosphamide caused a significant increase in the number of micronucleated PCEs 24 hours after application, thus validating the test.

Section A6.6.4 Genotoxicity in vivo

Annex Point IIA 6.6.4 **In-vivo bone marrow micronucleus study in mice**

		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Potential mutagenicity of Cypermethrin technical was examined in bone marrow of Swiss albino mice according to OECD test guideline 474. The *in vivo* micronucleus assay assesses the clastogenic and aneugenic potential of the test substance, which was administered orally at three dose levels; 50, 25 and 12.5 mg/kg. Following a range-finding study, the MTD was found to be 50 mg/kg. In the main study, animals were treated once via the oral route (corn oil used as the vehicle) and samples were taken 24 and 48 hours after treatment. One group of vehicle controls and a high dose group were treated on two occasions and samples collected 24 hours after the second dosing occasion. Cyclophosphamide was included as a standard positive control for the assay. During the microscopic evaluation, 2000 PCEs were scored per animal to assess the micronucleated cells. The PCE:NCE ratio was evaluated from assessment of 200 erythrocytes per group.	
5.2	**Results and discussion**	Single oral doses of 50, 25 or 12.5 mg/kg did not induce an increase in the frequency of micronucleated polychromatic erythrocytes (MCPEs) in male and female mice at 24 and 48 hours after treatment when compared to the vehicle control. The positive control induced a statistically significant increase in MPCE thereby validating the techniques used in the assay. The ratio of polychromatic to normochromatic erythrocytes was similar to the vehicle controls for the low and intermediate treatment regimen, although a slight reduction was evident for the treated groups at both sampling time points. The high dose group showed a reduction in the ratio also with a significantly lower ratio at 50 mg/kg compared to the vehicle control.	X
5.3	**Conclusion**	Cypermethrin technical induced no apparent chromosomal or other change leading to formation of micronuclei in polychromatic erythrocytes at dose levels up to the MTD of 50 mg/kg. Cypermethrin technical was not considered to be genotoxic and proved to be negative for mutagenicity in the mouse in-vivo bone marrow micronucleus test.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	January, 2011.
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	1
Acceptability	acceptable
Remarks	