CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

Substance Name:
cypermethrin (ISO); α-cyano-3-phenoxybenzyl 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate; cypermethrin cis/trans +/- 40/60

EC Number: 257-842-9

CAS Number: 52315-07-8

Index Number: 607-421-00-4

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Part A.

TEST SUBSTANCE RELATED EFFECTS SEEN AT THE HIGH DOSE LEVEL (50 MG/KG BW/D) INCLUDING REDUCED FOOD CONSUMPTION AND REDUCED BODY WEIGHT IN MALES AND FEMALESCOMPARAID TO CONTROLS, BUT NO SUBSTANCE RELATED MORTALITY WAS OBSERVED (TABLE 35). MINOR STATISTICALLY SIGNIFICANT FLUCTUATIONS SEEN IN HAEMATOLOGICAL PARAMETERS IN THE INTERIM AND 2 YEARS GROUPS, THESE LACKING ANY COMPOUND RELATED TRENDS AND THEREFORE NOT CONSIDERED TO BE OF TOXICOLOGICAL SIGNIFICANCE. INCREASED ACTIVITY OF P-NITROANISOLE-O-DEMETHYLASE ACTIVITY (LIVER PNOD) OBSERVED IN BOTH MALES AND FEMALES CONFIRMED THAT

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	cypermethrin (ISO); α-cyano-3-phenoxybenzyl 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate; cypermethrin cis/trans +/- 40/60
EC number:	257-842-9
CAS number:	52315-07-8
Annex VI Index number:	607-421-00-4
Degree of purity:	Min 920 g/Kg
Impurities:	See confidential annex

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	GHS07 GHS09 Warning Acute Tox. 4*; H302 Acute Tox. 4*; H332 STOT SE3; H335 Aquatic acute 1; H400 Aquatic chronic 1; H410 M acute; / M chronic; /
Current proposal for consideration by RAC	Acute Tox 4 oral Acute Tox 4 inhalation STOT RE2 (nervous system) M acute = 100 M chronic = 1000
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute Tox. 4; H302 Acute Tox. 4; H332 STOT RE2; H373 (nervous system) STOT SE3; H335 Aquatic acute 1; H400 Aquatic chronic 1; H410 M acute = 100 M chronic = 1000

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	Not classified	Not applicable	Not classified	
2.2.	Flammable gases	Not classified	Not applicable	Not classified	
2.3.	Flammable aerosols	Not classified	Not applicable	Not classified	
2.4.	Oxidising gases	Not classified	Not applicable	Not classified	
2.5.	Gases under pressure	Not classified	Not applicable	Not classified	
2.6.	Flammable liquids	Not classified	Not applicable	Not classified	
2.7.	Flammable solids	Not classified	Not applicable	Not classified	
2.8.	Self-reactive substances and mixtures	Not classified	Not applicable	Not classified	
2.9.	Pyrophoric liquids	Not classified	Not applicable	Not classified	
2.10.	Pyrophoric solids	Not classified	Not applicable	Not classified	
2.11.	Self-heating substances and mixtures	Not classified	Not applicable	Not classified	
2.12.	Substances and mixtures which in contact with water emit flammable gases	Not classified	Not applicable	Not classified	
2.13.	Oxidising liquids	Not classified	Not applicable	Not classified	
2.14.	Oxidising solids	Not classified	Not applicable	Not classified	
2.15.	Organic peroxides	Not classified	Not applicable	Not classified	
2.16.	Substance and mixtures corrosive to metals	Not classified	Not applicable	Not classified	
3.1.	Acute toxicity – oral	**Acute tox 4, H302**	Not applicable	**Acute tox 4*, H302**	
	Acute toxicity – dermal	Not classified	Not applicable	Not classified	
	Acute toxicity – inhalation	**Acute tox 4, H332**	Not applicable	**Acute tox 4*, H332**	
3.2.	Skin corrosion / irritation	Not classified	Not applicable	Not classified	
3.3.	Serious eye damage / eye irritation	Not classified	Not applicable	Not classified	
3.4.	Respiratory sensitization	Not classified	Not applicable	Not classified	
3.4.	Skin sensitization	Not classified	Not applicable	Not classified	
3.5.	Germ cell mutagenicity	Not classified	Not applicable	Not classified	
3.6.	Carcinogenicity	Not classified	Not applicable	Not classified	
3.7.	Reproductive toxicity	Not classified	Not applicable	Not classified	
3.8.	Specific target organ toxicity – single exposure	**STOT SE3, H335**	Not applicable	**STOT SE3, H335**	
3.9.	Specific target organ toxicity – repeated exposure	**STOT RE2, H373 (nervous system)**	Not applicable	Not classified	

3.10.	Aspiration hazard	Not classified	Not applicable	Not classified	
4.1.	Hazardous to the aquatic environment	**Aqu. acute 1, H400** **Aqu. chronic 1, H410**	**M acute=100** **M chronic= 1000**	**Aqu. acute 1, H400** **Aqu. chronic 1, H410**	
5.1.	Hazardous to the ozone layer	Not classified	Not applicable	Not classified	

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Pictograms :

GHS07

GHS08

GHS09

Signal word: Warning

Hazard statements:
H332 Harmful if inhaled
H302 Harmful if swallowed
H373 May cause damage to organs through prolonged or repeated exposure
H335 May cause respiratory irritation
H410 Very toxic to aquatic life with long lasting effects

Precautionary statements:
P-statements are not included in annex VI

Proposed notes assigned to an entry: /

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

The current entry for harmonised classification of cypermethrin was finalised during the meeting on pesticides –Health Effects of the Commission Working Group on the Classification and Labelling of Dangerous substances – Ispra 25-27 April 2001 and introduced in the 29 ATP (Directive 2004/73/EC) of the Annex I of Directive 67/548. The CLP Regulation (EC) N° 1272/2008, introduces new guidance values for Acute toxicity and for specific target organ toxicity-repeated exposure on one side, and introduces on a second side the M-factors for the classification for the toxicity of a mixture for the hazard to the environment. Considering that no new scientific arguments related to thoses aspects were introduced since the current classification was been established, it was agreed during the CARACAL – meeting of 09/07/2014 that a proposal focussing only on the end point to be revised can be submitted (acute toxicity oral and inhalation; STOT-RE)). Considering that no M-Factor is currently included in the Harmonised Classification for cypermethrin, a full proposal for the endpoints related to the environment is introduced.

Therefore, this proposal will focus on the revision of the **Acute toxicity and the Specific target organ toxicity-repeated exposure** classification and on **the setting of M-Factor for the environment** based on the available information in December 2015.

2.2 Short summary of the scientific justification for the CLH proposal

The current classification is a translation of the former classification for cypermethrine according to the 29th ATP of the former harmonised classification system. Since classification criteria have been changed in the current CLP regulation in comparison the former system, a validation of the classification has to be checked at least for the Acute Toxicity criteria marked with an (*).

The results from the 5-week and 90-day repeated dose toxicity studies in dogs justify classification of cypermethrin because the neurotoxicity effects observed at 37.5 mg/kg bw/day are between the classification cut off of 10<C ≤100 mg/kg bw/day for STOT RE2.

For the environment part of the classification, M-factor has been introduced as part of the classification and are needed in order to classify mixture or products. No M-factor exists in current classification and this need to be setted.

2.3 Current harmonised classification and labelling

Acute Tox. 4*; H332
Acute Tox. 4*; H302
STOT SE3; H335
Aquatic acute 1; H400
Aquatic chronic 1; H410

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Cypermethrine is an active substance already evaluated in the framework of the former Directive 98/8/CE (Biocide) for the PT 8 (wood preservatives). During the evaluation of the

active substance it was agreed that the current Harmonised Classification of cypermethrin will have to be revised. The submission of the draft Competent Authority Report (CAR) for the Product type 18 for cypermethrin under the regulation 528/2012 (biocide regulation replacing the Directive 98/8/CE) cannot be done without the delivery of the proposal for revision of CLH for cypermethrin.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

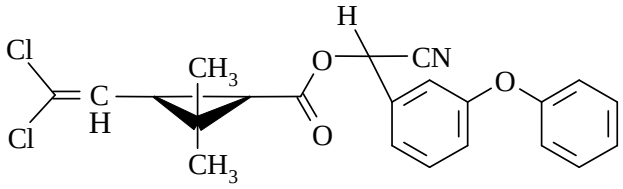
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	***257-842-9***
EC name:	***α-cyano-3-phenoxybenzyl 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate***
CAS number (EC inventory):	***52315-07-8***
CAS number:	***52315-07-8***
CAS name:	***Cyclopropanecarboxylic acid, 3-(2,2-dichloroethenyl)-2,2-dimethyl-, cyano(3-phenoxyphenyl)methyl ester***
IUPAC name:	***(RS)-α-cyano-3 phenoxybenzyl-(1RS)-cis, trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate***
CLP Annex VI Index number:	***607-421-00-4***
Molecular formula:	$C_{22}H_{19}Cl_2NO_3$
Molecular weight range:	***416.3***

Structural formula:



1.2 Composition of the substance

Isomer ratio cis:trans 40:60

Table 5: Detailed isomeric composition

Cis I	23.3%
Cis II	16.8%
Total Cis Isomers	40.1%
Trans I	35.8%
Trans II	24.1%
Total Trans Isomers	59.9%

Cypermethrin cis:trans isomer ratio 40(±5):60(±5).

The cypermethrin molecule has 3 chiral centers giving rise to 8 stereoisomers, four pairs of enantiomers – two cis (CIS I & CIS II) and two trans (TRANS I & TRANS II). Each enantiomeric pair is racemic – i.e. 50:50 mix of each enantiomer, as resumed in tables below.

Table 6: Constituents (non-confidential information only)

Constituent	Typical concentration	Concentration range	Remarks
Cypermethrin cis/trans +/- 40/ 60	/	**Min 920g/Kg**	

Table 7: Overview of the eight isomers of cypermethrin cis/trans +/- 40/ 60

	C.A. denomination of the isomers	CAS n°		Most common Cis-Trans ratios	
1	[1R-(1α(S*),3α)]	65731-84-2	cis-II	40% min	48% max
2	[1S-(1α(S*),3α)]	72204-44-5			
3	[1R-(1α(R*),3α)]	65731-83-1	cis-I		
4	[1S-(1α(R*),3α)]	72204-43-4			
5	[1R-(1α(S*),3β)]	65732-07-2	trans-II	60% max	52% min
6	[1S-(1α(S*),3β)]	83860-32-6			
7	[1R-(1α(R*),3β)]	66841-24-5	trans-I		
8	[1S-(1α(R*),3β)]	83860-31-5			

Table 8: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Impurities	See confidential annex		

Table 9: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
None				

1.2.1 Composition of test material

As stated above.

1.3 Physico-chemical properties

No modification as regard to the physic-chemical properties compared to the current classification is required.

Table 10: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Homogenous liquid	*B. de Ryckel*, 2005	
Melting/freezing point	Endotherm onset 41.2°C, peak 47.3°C	*Bates*, 2002a	
Boiling point	Boiling did not occurs, decomposition observed starting from approximately 200°C	*Bates*, 2002a	
Relative density	1.303	*Bates*, 2002a	
Vapour pressure	$6.4x10^{-7}$ Pa at 25°C	*Sydney*, 2005a	
Surface tension	25.6 mN/m (25°C) 24.2 mN/m (39.9°C)	*B. de Ryckel*, 2005	
Water solubility	< 9µg/L 4µg/L	*Bates*, 2002a *The pesticide manual, eleven Edition, Ed. CSD Tomlin, British Crop Protection Council*, 1997	
Partition coefficient n-octanol/water	Log Pow = 5.3-5.6	*Bates*, 2002a	
Flash point	>79°C	*B. de Ryckel*, 2005	
Flammability	Auto-ignition temp. = 385°C	*B. de Ryckel*, 2005	
Explosive properties	Not explosive	*B. de Ryckel*, 2005	
Self-ignition temperature	400°C	*Bates*, 2002b	
Oxidising properties	Not oxidising	*B. de Ryckel*, 2005	
Granulometry	Not relevant (liquid)	*B. de Ryckel*, 2005	
Stability in organic solvents and identity of relevant degradation products			
Dissociation constant	Not applicable		
Viscosity	No Newtonian flow behaviour. Dynamic viscosity at 20°C +/- 0.5°C was 15 mPa.s to 64 mPa.s depending on the shear rate applied to the sample.	*B. de Ryckel*, 2005	

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant in this dossier.

2.2 Identified uses

Cypermethrin cis/trans +/- 40/ 60 is a large spectrum insecticide intended to be used in Plant Protection Products and in Biocides.

It has to be pointed out that industries defending Cypermethrin cis/trans +/- 40/ 60 under PPP and under Biocides are differents actor (only one industry is defending Cypermethrin cis/trans +/- 40/ 60 under both biocides and PPP)) However, technical equivalence has been performed in both framework to unsure that a common assessment can be performed. It is therefore considered here that only Cypermethrin cis/trans +/- 40/ 60 is relevant for the CLH report even if cross reading or older studies with different or unknown isomeric composition are sometimes used to address data gaps or to provide supporting information.

Due to the isomeric toxicity, Cypermethrin with other isomeric composition than Cypermethrin cis/trans +/- 40 / 60, is not considered in this CLH dossier.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not evaluated in this dossier.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

Supportive data for STOT RE:

Table 11: List of ADE study supportive for STOT RE classification

Route	Species	Test substance	Dose (mg/Kg bw)	Method	Results	Reference
Oral GLP =Yes Rel = 1	Rat m/f balance study : 4/sex/group distribution study: 12/sex/group	**Cypermethrin cis:trans/40:60** ^{14}C-radiolabelled in cyclopropyl or phenyl ring	Balance study: single dose, 3 or 50 mg/Kg bw Distribution study: repeated: 9 days, 3 mg/Kg bw/d	OECD 417 Excretion balance study: rates and routes sampling urine: 6, 12, 24, 48, 72, 96, 120, 144h; faeces: 24, 48, 72, 96, 120, 144h; expired air: 24, 48h Tissue distribution study: concentration of radioactivity determined in the tissues at 24h, after1, 7, and 9 doses and at 7 days after 9 doses	Excretion Balance study *Low dose, 3 mg/Kg bw, [^{14}C-cyclopropyl]-cypermethrin* Excretion virtually complete by 72h after dosing. Mean overall recovery was 102.9 ± 2.6% of the dose. The excretion of radioactivity was split equally between the urine (47.8% of the dose in males, 52.9% in females) and faeces (50.2% in males and 43.4% in females). There was no significant elimination of [^{14}C]-carbon dioxide in the expired air (< 0.3% of the dose). The minimum absorption, as measured by the radioactivity excreted in the urine and cage washes and debris, was 52.8% of the dose the males and 57.6% in the females. The residual carcass contained < 0.7% of the dose showing that elimination of the radioactive dose was complete. *Low dose, 3 mg/Kg bw, [^{14}C-phenyl]-cypermethrin* Excretion virtually complete by 72h after dosing. The mean overall recovery of radioactivity was 101.4 ± 5.3%. The main route of excretion was *via* the faeces (48.5 and 59.8% of the dosed radioactivity in males and females respectively) with the urine containing a further 47.5% of the dose in the males and 40.8% in the females, though there was significant inter-individual variation. There was no significant elimination of [^{14}C]-carbon dioxide in the expired air (below the LOQ). The minimum absorption, as measured by the radioactivity excreted in the urine and cage washes and debris, was 51.3% of the dose in the males and 43.6% in the females. The residual carcass contained 0.4 - 0.6% of the dose showing that elimination of the radioactive dose was essentially complete.	[redacted] ***2006***

					High dose, 50 mg/Kg bw, [^{14}C-cyclopropyl]-cypermethrin Excretion virtually complete by 72h after dosing. The mean overall recovery was 106.0 ± 5.7% of the dose. The mean recovery values showed a slight sex difference in the excretion of the radioactivity with more of the dose being excreted in the urine of the females, though, there were significant inter-individual variations in the route of excretion. However, the main route of excretion was *via* the faeces (78.6 and 60.7% of the dosed radioactivity in males and females respectively), the urine contained a further 27.2% of the dose in the males and 36.9% in the females. There was no significant elimination of [^{14}C]-carbon dioxide in the expired air (< 0.2% of the dose). The minimum absorption, as measured by the radioactivity excreted in the urine and cage washes and debris, had fallen at the high dose to 28.7% of the dose in the males and 42.5% in the females. The residual carcass contained approximately 0.4% of the dose showing that elimination of the radioactive dose was complete. *High dose, 50 mg/Kg bw, [^{14}C-phenyl]-cypermethrin* Excretion virtually complete by 72h after dosing. The mean overall recovery was 109.7 ± 4.9% of the dose. The main route of excretion was *via* the faeces (80.0 and 68.3% of the dosed radioactivity in males and females respectively), with the urine containing a further 29.1% of the dose in the males and 34.8% in the females. There was no significant elimination of [^{14}C]-carbon dioxide in the expired air (below the limit of quantification). The minimum absorption, as measured by the radioactivity excreted in the urine and cage washes and debris, was 31.5% of the dose in the males and 38.4% in the females. The residual carcass contained 0.5% of the dose showing that elimination of the radioactive dose was essentially complete. <u>In conclusion, oral absorption:</u> Low dose 3 mg/Kg bw: group A ♂ 52.8% ♀ 57.6%; group C ♂ 51.3% ♀ 43.6% High dose 50 mg/Kg bw: group B ♂ 28.7% ♀ 42.5%; group D ♂ 31.5% ♀ 38.4%	

					At 144 h after dosing, the highest residues were found in the fat for all dose groups. **Tissue distribution study:** The highest levels of radioactivity were found in the fat (peri-renal, inguinal and subcutaneous) at all timepoints. Residues were rapidly cleared from the body once dosing had ceased. In males, the levels in the plasma 24h after nine doses (565.5 ng equivalents/g) were twice those seen 24h after a single oral dose. The highest increases (> 10-fold) in the concentration of radioactivity were measured in the inguinal and peri-renal fat. In these tissues, the concentrations of residues rose from 91.8 to 1009 ng equivalents/g in the case of the inguinal fat and from 197.5 to 1966 ng equivalents/g in the case of the peri-renal fat. The lowest levels of radioactivity were seen in the brain (< 9 ng equivalents/g) and spinal cord (< 36 ng equivalents/g). In female rats, the levels of radioactivity in the plasma were approximately 20% higher on day 10 (698.2 ng equivalents/g) than on day 2 (579.5 ng equivalents/g). The levels in the inguinal and peri-renal fat rose by 6-7 times those seen on day 2, the concentrations of residues rising from 204 to 1196 ng equivalents/g in the case of the inguinal fat and from 295 to 2179 ng equivalents/g in the case of the peri-renal fat. The lowest levels of radioactivity were seen in the brain and spinal cord (< 21 ng equivalents/g). The radioactivity in the tissues was rapidly cleared, and by day 16, 7 days after the last dose, many of the tissues contained levels of radioactivity that had fallen below the limit of detection. The concentrations of radioactivity in the fats had fallen by 2-6 times when compared to the levels on day 10 whilst the levels in the plasma had fallen by approximately 30 times.	

Toxicokinetics/Metabolism
ADE study in the rat

Reference	[redacted]. (2006); [^{14}C]-Cypermethrin-cis:trans 40:60:- Absorptin, Distribution and Excretion in the Rat; Covance Laboratories Limited, report no. 1669/029, 31 March 2006 (unpublished). Dates of experimental work: 14 April 2005 – 23 January 2006
Guideline study	Yes, OECD Guideline 417, OPPTS 870.7485 (1998)
GLP	Yes
Deviations	Yes Because of the nature of the formulation - a solution of cypermethrin in corn oil, a trial formulation to assess homogenicity, stability, and radioactivity concentration was not performed prior to the preparation of the formulations for dose administration. The formulation prepared for dose group A was subsequently used to determine homogeniceity and stability at 4 and 11 days after preparation. A number of rats were above the weight range (180-220g). The number, quantity and identity of radiolabelled metabolites in urine, faeces, and bile and a proposed metabolic pathway were not determined in the study.

- ***Materials and methods***

Test material	Cypermethrin cis:trans/40:60
Lot/Batch numbers	AS 175COV/05 (Cis Cypermethrin, non-radiolabelled) AS 176COV/05 (Trans Cypermethrin, non-radiolabelled)
Purity	98.4 % w/w (Cis Cypermethrin, non-radiolabelled) 98.9 % w/w (Trans Cypermethrin, non-radiolabelled)
Stability	Stable

Toxicokinetics/Metabolism
ADE study in the rat

Radiolabelling	Radiolabelled cypermethrin was supplied as separate cis- and trans-cypermethrin labelled in either the cyclopropyl or phenyl ring: Cis [^{14}C-cyclopropyl]-cypermethrin Study number 04 BLY 115b Specific radioactivity 53 mCi/mmol (4.7 MBq/mg) Radiochemical purity 100%. Trans [^{14}C-cyclopropyl]-cypermethrin Study number 04 BLY 115b Specific radioactivity 53 mCi/mmol (4.7 MBq/mg) Radiochemical purity 100%. Cis [^{14}C-phenyl]-cypermethrin Study number 04 BLY 115b Specific radioactivity 55.4 mCi/mmol (4.9 MBq/mg) Radiochemical purity 100%. Trans [^{14}C-phenyl]-cypermethrin Study number 04 BLY 115b Specific radioactivity 55.4 mCi/mmol (4.9 MBq/mg) Radiochemical purity 100%.
Species	Rat
Strain	Sprague Dawley (Crl:CD®(SD) IGSBR)
Source	Charles River (UK) Ltd
Sex	Males and Females
Age/weight at study initiation	Males: 188 – 301 g Females: 180 – 247 g
Number of animals per group	4 males, 4 females (excretion balance studies) 12 males, 12 females (tissue distribution study) See Table A6.2_01-1
Control animals	No
Administration/ Exposure	Oral
Type	Gavage
Concentration of test substance	See Table
Preparation of test substance	Appropriate amounts of the non-radiolabelled *cis* and *trans* isomers were weighed into a formulation vessel. Appropriate volumes of the *cis* and *trans* isomers of [^{14}C]-cypermethrin were then transferred to the same formulation vessel which was agitated to co-dissolve the non-radiolabelled test substance. The solvent was removed under a stream of nitrogen and an appropriate volume of corn oil was then added to the test substances, which were dissolved using sonication or mixing.
Dose volume	5 ml/kg

Toxicokinetics/Metabolism
ADE study in the rat

Dose administration	Single doses were administered in the balance study. In the distribution study, doses were administered daily for up to 9 days
Sampling time	See Table A6.2_01-1 At each collection timepoint, cage debris was removed and the cages washed with water and then a methanol wash. Cage debris and washings were pooled separately for each animal.
Tissue analysis	**Balance studies** Following the final sample collection, the animals were exsanguinated by cardiac puncture under anaesthesia and weighed blood samples were taken into two heparin-lined tubes, one of which was used to prepare plasma. The residual carcasses and the following tissues were also taken for analysis: Adrenals, bone, brain, fat, GI tract (+ contents) heart, kidneys, liver, lungs, muscle (quadriceps), ovaries and uterus (females only), skin, spleen, testis (males only). **Distribution study** Animals were killed by cold shock in a mixture of hexane and solid carbon dioxide following deep anaesthesia. Carcasses were retained in the freezing mixture for at least 30 mins and were then stored frozen (-20ºC) before being prepared for QWBA. Blood samples were also taken prior to terminal sacrifice in order to prepare plasma.
Treatment of samples	Carcasses were digested in potassium hydroxide (40% solution in methanol) under reflux. Digests were neutralised prior to LSC analysis. Blood samples were incubated with solubilising agent. Liquid scintillant was then added and the samples left to dark-adapt prior to LSC analysis. Faeces, cage debris and tissues were similarly treated with solubilising agent and left to incubate before the addition of liquid scintillant. Quantitative Whole body Autoradiograpy (QWBA) Legs, whiskers and tail were trimmed off and each frozen carcass was set in a block of aqueous 2% (w/v) carboxymethylcellulose. Sagittal sections (nominal thickness 30 µm) were obtained at a minimum of 5 levels through the carcass using a cryomicrotome. These levels included, but were not limited to, the following tissues: exorbital lachrymal gland (males) or ovary (females), intra-orbital lachrymal gland, Harderian gland, adrenal gland, thyroid, brain and spinal cord. The sections were mounted, freeze-dried and placed in contact with FUJI imaging plates. [^{14}C]-Blood standards of appropriate activity (also sectioned at a nominal thickness of 30 µm) were placed in contact with all imaging plates and exposed for 7 days in a copper lined lead exposure box. After exposure, the imaging plates were processed using a FUJI FLA 5000 radioluminography system. The carbon-14 blood standards included with each autoradiogram were used to construct calibration lines over a range of radioactivity concentrations.
• ***Results and discussion***	At the higher dose level, faecal excretion was the major route of elimination accounting for 79 and 61% when [^{14}C-cyclopropyl]-cypermethrin was dosed and 80 and 68% when [^{14}C-phenyl]-cypermethrin was dosed. In each case, the higher excretion level

Toxicokinetics/Metabolism
ADE study in the rat

was seen in the male rats (Table 13). The observed increase in faecal elimination suggests that the absorption process was being saturated at the higher dose level.

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

Only trace amounts of radioactivity were measured in the expired carbon dioxide confirming that the positions of radiolabel were metabolically stable in the rat (Table 13).

At necropsy, 144 h after dosing, the levels of radioactivity measured in the tissues generally reflected the lipophilic nature of cypermethrin with the highest levels being found in the fat in all dose groups. These levels were approximately 6 times higher than any other tissue in the case of the male rats. In female rats, the ovaries generally contained the next highest concentrations of radioactivity, approximately 3 times lower than those seen in the fat (Table 14).

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

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

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

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

Toxicokinetics/Metabolism
ADE study in the rat

- ***Conclusion*** Excretion of radioactivity was virtually complete by 72 h following a single oral dose of [^{14}C-cyclopropyl]- or [^{14}C-phenyl]-cypermethrin at a dose rate of 3 or 50 mg/kg bodyweight. Urinary and faecal excretion were similar at the low dose for both radiolabels, but at the higher dose level faecal excretion predominated, especially in the males. This suggests that the absorption of cypermethrin was being saturated at the high dose rate. As minimum of 43.6-57.6% of the dose was absorbed at the low dose level. At the high dose level, a minimum of 28.7 to 31.5% of the dose was adsorbed in male rats and 38.4 to 42.7% in the case of the females. At 144 h after dosing, the highest residues were found in the fat for all dose groups.

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

Reliability 1

Deficiencies none

Table 12: Treatment schedule and dose rates ([redacted], 2006)

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

Table 13: Overall recovery (mean % of administered dose)– Ex. Bal. study

Excreta	Time-point (h)	Low Dose (3 mg/kg bw)				High Dose (50 mg/kg bw)			
		[^{14}C]-cyclopropyl (Group A)		[^{14}C]-phenyl (Group C)		[^{14}C]-cyclopropyl (Group B)		[^{14}C]-phenyl (group D)	
		Males	Females	Males	Females	Males	Females	Males	Females
Urine	6	1.163	2.676	5.686	4.203	3.242	1.071	5.265	3.873
Urine	12	15.640	23.301	15.771	14.768	5.415	0.152	8.983	9.404
Urine	24	18.396	18.897	20.444	14.215	10.641	0.112	11.306	14.554
Urine	48	9.674	5.807	4.474	6.623	5.731	0.836	2.818	6.079
Urine	72	1.894	0.983	0.685	0.581	1.393	1.023	0.393	0.494
Urine	96	0.675	0.850	0.242	0.220	0.468	0.283	0.183	0.215
Urine	120	0.281	0.293	0.131	0.134	0.162	0.209	0.105	0.121
Urine	144	0.125	0.130	0.108	0.077	0.100	0.108	0.064	0.079
	Subtotal	47.846	52.935	47.541	40.822	27.150	36.883	29.115	34.816
Faeces	24	41.522	39.353	39.000	46.737	72.486	51.200	72.090	62.094
Faeces	48	7.135	3.305	6.269	9.980	4.849	7.366	6.354	5.808
Faeces	72	1.019	0.444	1.789	2.218	0.803	1.071	1.380	0.225
Faeces	96	0.318	0.154	1.312	0.161	0.291	0.152	0.241	0.094
Faeces	120	0.148	0.085	0.101	0.694	0.130	0.112	0.044	0.045
Faeces	144	0.095	0.050	0.042	0.016	0.070	0.836	0.020	0.011
	Subtotal	50.236	43.389	48.512	59.869	78.629	60.737	80.127	68.275
Cage Wash + Debris		4.992	4.624	3.717	2.773	1.591	5.660	2.352	3.582
CO_2 Traps		0.255	0.151	BLQ	BLQ	0.121	0.164	BLQ	BLQ
Carcass	144	0.680	0.523	0.584	0.338	0.462	0.410	0.469	0.417
G.I. Tract +Contents	144	0.133	0.115	0.027	BLQ	0.068	0.056	0.083	0.110
	Subtotal	0.813	0.637	0.611	0.338	0.530	0.466	0.553	0.527
Overall recovery		104.143	101.735	100.381	102.487	108.023	103.91	112.145	107.198

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

Table 14: Mean concentration of radioactivity in tissues (ng equivalents/g tissue) – Ex. Bal

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

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

Table 15: Mean concentration of radioactivity in tissues -Repeated dose study, male rats

	Mean Concentration of [^{14}C]-Cypermethrin residues (ng equivalents/g tissue)			
Number of doses given	**1**	**7**	**9**	**9**
Kill time	**Day 2**	**Day 8**	**Day 10**	**Day 16**
Plasma	292.9	432.5	565.5	17.90
Blood	182.2	241.7	315.4	BLQ
Aorta	193.7	292.7	540.6	BLQ
Mandibular lymph nodes	53.57	131.6	323.6	91.97
Kidney cortex	231.4	545.9	652.5	78.71
Kidney medulla	137.6	361.0	430.3	BLQ
Liver	180.7	455.8	693.9	88.29
Brain	BLQ	7.05	8.18	BLQ
Pineal body	92.67	BLQ	167.16	BLQ
Spinal cord	BLQ	21.04	35.83	BLQ
Adrenal	129.7	753.1	686.7	291.0
Pituitary	BLQ	293.2	58.83	BLQ
Thymus	23.50	38.56	48.11	BLQ
Thyroid	104.2	184.4	427.2	45.10
Exorbital lachrymal gland	45.51	80.49	109.1	BLQ
Harderian gland	93.52	255.3	224.2	10.31
Intra-orbital lachrymal gland	40.75	149.2	157.2	53.49
Salivary glands	35.73	62.17	100.3	19.42
Brown fat	568.8	1565	1936	321.1
Inguinal fat	91.81	953.4	1009	581.0

Peri-renal fat	197.5	1319	1966	717.7
Subcutaneous fat	86.63	381.9	351.6	73.48
Bulbo-urethral gland	43.18	73.71	116.10	BLQ
Epididymis	43.05	209.5	102.2	193.8
Preputial gland	82.20	431.8	635.4	367.4
Prostate	56.29	119.3	170.2	190.4
Seminal vesicles	48.82	31.99	366.6	204.3
Testis	41.87	59.87	65.82	8.18
Muscle	21.42	30.50	36.82	BLQ
Myocardium	59.39	102.0	127.5	BLQ
Tongue	53.84	81.50	128.7	BLQ
Skin	79.05	224.5	208.7	99.74
Uveal tract	76.22	76.27	132.9	BLQ
Bone marrow	32.79	76.27	62.71	29.98
Lung	136.4	195.4	260.0	7.82
Pancreas	43.21	98.76	72.81	20.56
Spleen	36.80	66.18	369.2	12.43
Tooth pulp	51.06	91.33	164.1	BLQ
Nasal mucosa	104.3	141.8	201.2	28.12
Oesaphagus wall	71.57	170.7	130.1	BLQ
Stomach mucosa	52.77	130.3	184.6	BLQ
Small intestine mucosa	270.8	650.6	471.5	BLQ
Caecum mucosa	167.8	321.3	1232	126.8
Large intestine mucosa	488.2	1202	2490	52.51
Rectum mucosa	111.4	599.8	584.6	BLQ
Limit of Quantification	23.50	22.11	21.75	22.01

BLQ - Tissue measurement below lower limit of quantification
NA - Not Available

Table 16: Mean concentration of radioactivity in tissues -Repeated dose study, female rats

	Mean Concentration of [^{14}C]-Cypermethrin residues (ng equivalents/g tissue)			
Number of doses given	**1**	**7**	**9**	**9**
Kill time	**Day 2**	**Day 8**	**Day 10**	**Day 16**
Plasma	579.5	548.4	698.2	24.40
Blood	381.1	344.0	452.9	22.41
Aorta	416.7	462.3	493.5	BLQ
Mandibular lymph nodes	117.3	145.2	154.8	52.88
Kidney cortex	439.6	656.4	926.1	127.2
Kidney medulla	276.9	167.7	238.5	26.22
Liver	651.6	882.9	991.8	103.3
Brain	7.744	9.187	15.54	BLQ
Pineal body	131.5	127.1	96.67	BLQ
Spinal cord	8.279	8.706	20.14	BLQ
Adrenal	220.3	342.9	784.1	358.9
Pituitary	78.67	NA	77.07	19.12
Thymus	36.21	37.97	384.3	BLQ
Thyroid	149.1	139.9	285.2	7.424
Harderian gland	78.99	138.1	193.9	23.93
Intra-orbital lachrymal gland	107.1	127.1	189.3	57.42
Salivary glands	72.32	91.06	152.6	8.599
Brown fat	882.9	1176	1831	280.9

Inguinal fat	203.7	1032	1196	347.2
Peri-renal fat	294.6	1250	2179	705.5
Subcutaneous fat	156.4	385.6	385.1	151.4
Clitoris	140.0	242.7	346.1	64.89
Ovary	247.8	837.5	1042*	715.2*
Uterus	233.1	549.7	943.8	BLQ
Muscle	26.44	23.39	34.66	BLQ
Myocardium	121.2	102.6	161.4	8.439
Tongue	102.5	109.4	150.3	BLQ
Skin	145.5	626.5	619.6	339.7
Uveal tract	166.2	166.6	229.1	BLQ
Bone marrow	92.24	212.0	198.6	111.9
Lung	255.3	258.0	322.1*	30.60
Pancreas	81.24	88.45	201.3	15.65
Spleen	48.60	NA	100.6	BLQ
Tooth pulp	163.2	148.1	236.6	BLQ
Nasal mucosa	168.5	127.9	370.9	51.75
Oesaphagus wall	103.3	210.8	275.4	BLQ
Stomach mucosa	209.4	157.3	341.3	BLQ
Small intestine mucosa	197.1	190.6	1473	BLQ
Caecum mucosa	556.0	562.9	591.8	BLQ
Large intestine mucosa	731.2	733.3	1344	BLQ
Rectum mucosa	1056	195.5	1764	BLQ
Limit of Quantification	23.23	25.96	22.11	22.11

BLQ – Tissue measurement below lower limit of quantification
NA – Not Available
* - Measurement affected by high levels of radioactivity in surrounding fat or tissue

4.2 Acute toxicity

4.2.1 Summary and discussion of acute toxicity findings relevant for classification as ACUTE TOX according to CLP Regulation

The following information was extracted from the toxicology chapter of the assessment report prepared for Cypermthrin under Biocidal Product Regulation (BPR), from the DAR made for cypermethrin under PPP Regulation, and from open literature publications.

Results exposed below are acute toxicity results for cypermethrin cis:trans/40:60 only. Results obtained for other isomer ratio inducing a change in substance chemical name and/or index number are not relevant for classification purpose since not related to the same substance. An exception is made for a new available inhalation study ([redacted]., 2005) performed with cypermethrin (cis:trans/53:47) in the vehicle DMSO. This study is discussed here because, the only other reliable study available ([redacted]., 1985) was performed with cypermethrin (cis:trans/40:60) in the vehicle ethanol. In addition, the study has only a reliability 2 as the study was performed before GLP was compulsory. The [redacted] study (2005) has to be seen as supplementary.

In addition for acute oral toxicity, results exposed below are for the vehicles with the most potency effect, namely oil vehicles. It was discussed in the toxicology chapter of the assessment report prepared for Cypermethrin under Biocidal Product Regulation (BPR) that the oral toxicity of cypermethrin varies with the type of vehicle used. In general, aqueous suspensions were the least toxic and non-polar solutions the most toxic.

According to the current Harmonised Classification, the dermal route does not need to be re-evaluated.

In the table below, key studies are reported on a light grey background whereas supportive study are reported on a white background.

Table 17: List of acute toxicity studies

Route	Method Guideline	Test substance	Species Strain Sex no/group	Dose levels duration of exposure	Value LD50/LC50	Reference
Oral	OECD 423 GLP= yes Rel= 1	**Cypermethrin cis:trans/40:60** Purity 94% CMN92T1197AN	Rat: Wistar female 3/group	300, 2000 mg/Kg bw in refined **groundnut oil**, 15 days post exposure period	**500 mg/Kg bw (f)**	[redacted], 2005
Oral	Similar to OECD 401 GLP= no Rel= 2	**Cypermethrin cis:trans/40:60** Purity 92.6% CGA 55186 tech	Rat: Tif:RAIf (SPF) male/female 5/sex/group	300, 600, 1200, 2500, 5000 mg/Kg bw in **arachis oil**; 14 days post exposure period	**1732 mg/Kg bw (m)** 2150 mg/Kg bw (f) 1945 mg/Kg bw (m,f)	[redacted], 1984a
Oral	No GLP=no Rel= 3	**Cypermethrin cis:trans/37:63** Purity 92.4% NRDC 149 tech	Rat : Wistar male 10/group	Pre-treatment i.p. with corn oil 1 h before gavage. Dose levels: not mentioned, vehicle: **corn oil**.	**250 mg/Kg bw (m)**	*Cantalamessa F.*, 1993
Inhalation	OECD 403 GLP= Yes REL= 1	**Cypermethrin Technical cis:trans/53:47** Purity 94%	Rat: wistar male/female 5/sex/group	0, 3.56 mg/L in DMSO; Mist, 4h, head and nose ; 15 days post exposure period	> **3.56 mg/l**	[redacted]., 2005
Inhalation	OECD 403 GLP= no REL= 2	**Cypermethrin cis:trans/40:60** Purity 92.6% **CGA 55186 tech**	Rat: Tif:RAIf (SPF) male/female 5/sex/group	0, 970, 1926, 3462, 5328 mg/m³; in ethanol; aerosol, 4h, nose only; 14 days post exposure period	**3281 mg/m³ (m)** **5038 mg/m³ (f)** **3894 mg/m³ (m,f)**	[redacted], 1985

Oral

Acute Oral Toxicity – Rat(1)

Rat, cypermethrin, oral LD50

Reference	[redacted]2005; Acute oral toxicity study (acute toxic class method) with cypermethrin in Wistar rats; Rallis Research Centre, India, report no. 4242/05, 15 July 2005 (unpublished).

Acute Oral Toxicity – Rat(1)

Rat, cypermethrin, oral LD50

Dates of work: 29th March 2005 – 22nd April 2005

- ***Materials and methods***

Guideline study	Yes, OECD guideline 423 (17th December 2001)
GLP	Yes
Deviations	No
Test material	Cypermethrin technical (cypermethrin cis:trans/40:60), vehicle : refined groundnut oil
Lot/Batch number	CMN92T1197AN
Purity	94.0% w/w
Study description	Acute oral administration in rat (acute toxic class method) with 15 day post-treatment observation is based on OECD guideline 423. A dose of 300 mg/kg bw (G1F group) in groundnut oil was administered to 3 female rats on day 1. Three further animals were treated at the same dose 3 days later. Based on the results, the next upper dose of 2000 mg/kg bw was administered to 3 animals to determine the LD50 cut-off value.
Controls	No
Examinations	Toxic signs and mortality observed 5 times during day 1 (at 30 mins and 4 times at hourly intervals) and once daily during days 2-15. Bodyweights are recorded pre-administration (day 1) and 8, 15 days post treatment/death. Gross necropsy performed at death and at the end of the observation period for all survivors.

- ***Results and discussion***

Clinical signs	No toxic signs/pre-terminal deaths in the G1F (300 mg/kg) group were detected. In the G2F (2000 mg/kg) group, toxic signs observed were slight/severe salivation, tremors, lethargy, ataxia and perineum wet with urine.
Pathology	In the G2F group, one rat died on day 2 and no abnormality was detected at necropsy. The other 2 rats died on day 3, lung congestion was detected in both rats at necropsy. No abnormalities detected in remaining rats (G1F) at necropsy.

Acute Oral Toxicity – Rat(1)

Rat, cypermethrin, oral LD50

Other	In the G1F group, all rats gained bodyweight during the observation period. In the G2F group, all dead rats lost weight compared to their initial bodyweight.
LD50	LD50 was found to be **500 mg/kg bw** as per LD50 cut-off value of Annex 2c of the guideline. Category 4 as defined by Globally Harmonised Classification system of Annex 2c of the guideline.
• ***Conclusion***	An LD50 of 500 mg/kg was determined based on the acute toxic class method.
Reliability	1
Deficiencies	No

Acute Oral Toxicity – Rat (2)

Rat, cypermethrin, oral LD50

Reference	[redacted], 1984; Acute Oral LD50 in the Rat of CGA 55186 Tech. (cypermethrin) – (administration in oily medium); Ciba-Geigy Ltd, report No.:840042 (CYP/T82b), 9 April 1984 (unpublished) Dates of work: 13 February 1984 – 2 May 1984
• ***Materials and methods***	
Guideline study	Existing study partially conforming to 92/69/EEC (OECD 401)
GLP	No, not existing in 1984 but Q.A. was made during the study
Deviations	Protocol partially conforms to OECD 401 (EC method B1) but with limited enquiries
Test material	CGA 55186 tech (cypermethrin cis:trans/40:60), vehicle : arachis oil
Purity	92.6%
Study description	The oral toxicity of cypermethrin was tested in the rat (Tif:RAIf (SPF), F3-crosses of RII 1/Tiff x RII 2/Tif) on 5 males and 5 females per dose level, aged of 7-8 weeks, weighting 162-225g. Animals were fed by gavage: 5000, 2500, 1200, 600, 300 mg/kg bw food consumption per day - ad libitum in Arachis oil Ph.H.VI Siegfried AG.

Acute Oral Toxicity – Rat (2)

Rat, cypermethrin, oral LD50

Controls	None
Examinations	Mortality recorded twice daily (once daily on weekends), clinical observations daily, body weight recorded on days 1, 7, 14 and at death. Gross necropsy performed at death and at the end of the observation period for all survivors.
Method of determination of LD_{50}	Logit method (J. Berkson, J.Am.Stat. Ass. 39. 357-65, 1944): LD50 including the 95% confidence limit

- ***Results and Discussion***

Clinical signs	Dyspnoea, exophthalmus, ruffled fur and curved body position was observed in all test groups. Diarrhoea and tremor were also observed. Tonic clonic convulsion was observed from 600 mg/kg bw onwards. At higher concentrations also salivation, sedation and lateral/ventral body positions were observed. Surviving animals recovered within 10 to 12 days. Animals died during the first 3 days post-exposure.
Pathology	Autopsies showed no gross lesions in the three lowest dose groups (300, 600, and 1200 mg/kg bw groups). In the 2500 mg/kg bw group one female had nasal discharge. In the 5000 mg/kg bw group a reddish and mottled lung was found and one animal had a dilated small intestine.

- ***Conclusion***

LD_{50}	LD50 in males 1732 (1027-2922) mg/kg bw LD50 in females 2150 (1342-4024) mg/kg bw LD50 in both sexes 1945 (1449-2676) mg/kg bw
Reliability	2

Acute Oral Toxicity – Rat (3)

Rat, cypermethrin, oral LD50

Reference	*Cantalamessa, F*, 1993; Acute toxicity of two pyrethroids, permethrin and Cypermethrin, in neonatal and adult rats. Arch Toxicol (1993) 67: 510-513 (published)
Guideline study	No, a specific investigation to determine the relative toxicity of a type I and type II pyrethroid when administered on a single occasion by the oral route to neonatal and adult rats. The study was also designed to assess pyrethroid biotransformation through the use of drug metabolism inhibitors and the effects on cypermethrin and permethrin toxicity.
GLP	No
• ***Materials and methods***	
Test material	Cypermethrin (type II pyrethroid) and permethrin (type I pyrethroid)
Specification	Technical grade cypermethrin – 62.8:37.2 *trans:cis*, 92.4% purity, vehicle: corn oil Technical grade permethrin – 75:25 *trans:cis*, 94% purity The two test materials investigated were technical grade cypermethrin (62.8:37.2 *trans:cis*, purity 92.4%) and technical grade permethrin (75:25 *trans:cis*, purity 94%). Wistar rats, pups and adults were bred in-house, the pups were maintained with their dams until weaning at 21 days. Pups of both sexes were used in the study but only male adult rats were treated. Prior to the single oral treatment (5ml/Kg bw), the adults were fasted for 16 h, the pups of 8 days were removed from the dam for a period of one hour and those of 16 or 21 days of age were removed for two hours. Food was returned to the adults immediately after dosing and the pups were returned to the mothers immediately following treatment. Ten animals per dose level were used to determine the LD_{50} values for the various age groups. The test substances were both dissolved in corn oil and administered in a dose volume of 5 ml/kg bw. Controls received the vehicle alone. Pre-treatment groups involved intraperitoneal administration of corn oil (1 h pre-dose - controls), TOPT the esterase inhibitor (125 mg/kg, 18 h pre-dose) or PB, monooxygenase inhibitor (150 mg/kg, 1 h pre-dose) All rats were observed regularly following dose administration and a comparison of PB/TOPT pre-treated animals with corn oil pre-treated control. The effects of the enzymatic inhibitors on the acute toxicity of the two test pyrethroids was investigated by using differing pre-treatment regimen and administering doses of cypermethrin or permethrin at their respective median lethal dose.

Acute Oral Toxicity – Rat (3)

Rat, cypermethrin, oral LD50

Statistical analysis of the median lethal dose was according to Thompson and Weil.

In the phase of the study using inhibitors the significance of differences in mortality between variously pre-treated groups was assessed using the Fischer chi^2 test.

- ***Results and Discussion***

A single oral administration of cypermethrin and permethrin to neonatal and adult rats showed that cypermethrin is more toxic than permethrin to adult and neonatal rats. It was noted that the sensitivity of rats to both test materials was greater the younger is the animal. Use of monooxygenase inhibitor or esterase inhibitor (pre-treatment of rats aged 8, 16 or 21days) did not cause any variation in the lethal effects of both test materials in neonatal rats. In the adult rats similarly pre-treated there was a significant increase in toxicity for both test materials in adults treated with esterase inhibitors but no increase in toxicity after treatment with the monooxygenase inhibitor.

It is postulated that the greater sensitivity in neonates reflects incomplete development of the enzymes that catalyse pyrethroid metabolism in the liver. Ester hydrolysis is suggested as an important mechanism for pyrethroid detoxification in adults.

Median lethal doses for cypermethrin (24 h LD_{50}; mg/kg bw):

8 days	14.9 (12.5-17.7)
16 days	27.1 (23.7-31.0)
21 days	49.3 (39.9-60.7)
Adult	250.0 (233.3-277.3)

Median lethal doses for permethrin:

8 days	340.5 (308.8-375.6)
16 days	399.0 (346.1-460.0)
21 days	471.0 (384.5-577.0)
Adult	1500.0 (938.0-2345.3)

The median lethal dose for cypermethrin was in the range of 15-50 mg/kg bw for neonates and some five-fold higher for adults, 250 mg/kg bw.

For permethrin the neonate range was notably higher than for cypermethrin, circa 340-470 mg/kg bw and for the adults the median lethal dose was three-fold higher than the weanling value.

The toxicity and sensitivity of neonates in the 8-16 day range was markedly higher for cypermethrin.

The type I pyrethroid permethrin induced a typical T syndrome response after 120-150 minutes, whereas the type II cypermethrin induced a CS response within 90-120 minutes. Both responses typically result in death.

Profuse salivation was evident among the adult rats following cypermethrin treatment, but not among the young pups (8 or 16 days old) and only sporadically among the weanlings (21 day old). Pre-treatment with TOPT and PB brought forward the onset of this response and also increased the intensity of the reaction.

Acute Oral Toxicity – Rat (3)

Rat, cypermethrin, oral LD50

	Pre-treatment with PB or TOPT did not produce a significant variation in lethality response in cypermethrin treated neonates (8, 16 or 21 days) but mortality was significantly increased among the adults pre-treated with the esterase inhibitor, although a similar effect was not apparent for PB. Pre-treatment of adult rats with TOPT similarly increased the percent mortality but the young rats (8, 16 or 21 days) were unaffected. Pre-treatment with PB had no effect on mortality for any of the adult or neonate rats.
• *Conclusion*	The greater sensitivity of neonatal rats to pyrethroid toxicity was attributed to incomplete development of the enzymatic systems responsible for catalyzing pyrethroid metabolism. Ester hydrolysis was identified as an important detoxification pathway in the adult rat for both cypermethrin and permethrin. Cypermethrin(24 h LD_{50}; mg/kg bw): 250.0 (233.3-277.3) Permethrin(24 h LD_{50}; mg/kg bw): 1500.0 (938.0-2345.3)
Reliability	3
Deficiencies	No

[redacted]., (2005) tested the oral toxicity, in the rat (Wistar) on 3 females per dose level, aged 9-10 week, weighing 132-152g. Animals were fed with 300mg/Kg bw and 2000mg/Kg bw test substance in refined groundnut oil. The LD50 cut-off value was determined with the acute toxic class method. No other information on statistics is available. Mortality was observed 2 days (1 rat) and 3 days (2 rats) after dosing in the 2000 mg/Kg bw group. Lung congestion was observed at necropsy in both rats that died on day 3. Tremors were observed in one rat of the 300 mg/Kg group. In the 2000 mg/Kg bw group, toxic clinical signs observed were slight/severe salivation, tremors, lethargy, ataxia, and perineum wet with urine. The LD50 was found to be 500 mg/Kg. The clinical signs observed were indicative for an action on the central nervous system and consisted of sedation, ataxia, splayed gait, salivation, tremors and convulsions (Table18). These signs appear within 1 hour after dosing and survivors recovered within 10-12 days.

Table 18: Acute oral toxicity result from [redacted], (2005) study

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

[redacted], (1984a) tested the oral toxicity in the rat (Tif:RAIf (SPF), F3-crosses of RII 1/Tiff x RII 2/Tif) on 5 males and 5 females per dose level, aged of 7-8 weeks, weighting 162-225g. Animals were fed by gavage: 5000, 2500, 1200, 600, 300mg/kgbw food consumption per day - ad libitum in Arachis oil Ph.H.VI Siegfried AG. LD 50 was determined by Logit method (J. Berkson, J.Am.Stat. Ass. 39. 357-65, 1944):LD50 including the 95% confidence limit. No further details on statistical analysis is available in the study. Mortality was observed 1 day after dosing, starting from 1200 mg/Kg bw (1 ♂, Day1), to 2500mg/Kg bw (3 ♂ ; 4 ♀, Day1) and 5000 mg/Kg bw (3♂; 4♀, Day1 and 2♂, 1♀; Day 3) (Table 19). Dyspnea, exophthalmus, ruffled fur and curved body position was observed in all test groups. Diarrhea (at 600 and 2500 mg/Kg 1 occurrence each) and tremor (at 1200 and 5000mg/Kg , 1 occurrence each) were also observed. Tonic clonic convulsion was observed from 600 mg/Kg bw onwards. At higher concentrations also salivation, sedation and lateral/ventral body positions were observed but the number of animal suffering from these symptoms are not reported. Instead, a degree of intensity is given which is for all symptoms described as slight. Surviving animals recovered within 10 to 12 days. At necropsy, one female of the 2500 mg/Kg bw group was found with nasal discharge. In the 5000 mg/Kg bw group, 1 reddish and mottled lung was observed and 1 animal had a dilated small intestine. LD_{50} =1732 (1027-2922) mg/kg bw in males; LD50 in females 2150 (1342-4024) mg/kg bw; LD50 in both sexes 1945 (1449-2676) mg/kg bw.

Table 19: Number and day of death in Acute oral toxicity study from [redacted]. (1984a)

Dose [mg/kg]	Number of dead / number of investigated	Time of death (range)
Males		
300	0/5	-
600	0/5	-
1200	1/5	D1
2500	3/5	D1
5000	5/5	D1 (3) or D3 (2)
Females		
300	0/5	
600	0/5	
1200	0/5	
2500	4/5	D1
5000	5/5	D1 (4) or D3 (1)

Cantalamessa F.,(1993) reported in a short communication (not a full study) an oral LD_{50} = 250 mg/Kg bw (rat, corn oil) for cypermethrin 37:63. While investigating the acute toxicity of cypermethrin in neonatal rats, the acute toxicity of cypermethrin (in corn oil) was reevaluated in male Wistar rats, using a control group and 4 dose groups (not given). Percent mortality was determined at 24 h and values of LD_{50} were calculated according to the method of *Thompson and Weil,* (1952). 90-

120 min after administration, rats exhibit a syndrome consisting of pawing and burrowing, facial licking and grooming at low doses, and at high doses uncoordinated movements, coarse tremors progressing to choreoathetosis, clonic seizure and death. Few details are available in the study. Initial doses are not provided, only mean results without further explanaition are stipulated.

Apart from the isomer ratio, the oral toxicity of cypermethrin varies with the type of vehicle. In general, as discussed in the assessment report prepared for Cypermethrin under Biocidal Product Regulation (BPR), aqueous suspensions were the least toxic and non-polar solutions the most toxic. Oral LD50 values vary from 250 mg/kg bw (in oil) to > 5000 mg/kg bw (aqueous solutions). The Wistar rat was found more sensitive than the Tif:RAIf (SPF) rat strain. The most relevant and reliable studies used as key studies in the assessment report prepared for Cypermethrin under Biocidal Product Regulation (BPR), are the same studies discussed here in the CLH report. All key studies are studies with cypermethrin administered in an oil as vehicle. Corn oil was found the vehicle with the highest potential potency.

Inhalation

Acute Inhalation Toxicity – Rat (1)

Rat, cypermethrin, Inhalation Exposure (LC50)

Reference	[redacted]. (2005); Acute Inhalation Toxicity Study with Cypermethrin Technical in wistar rats.
Guideline study	OECD 403
GLP	Yes
Deviations	No
• ***Materials and methods***	
Test material	cypermethrin technical (cypermethrin cis :trans/53 :47)
Lot/Batch number	CMN92T1197AN
Purity	94%

Acute Inhalation Toxicity – Rat (1)

Rat, cypermethrin, Inhalation Exposure (LC50)

Concentrations	Analytical average concentration: 0 mg/L (G1) 3.56 mg/L (G2)
Mean particule size	1.11+/- 0.67 µm (G1) and 1.23+/- 0.70 µm (G2)
Type or preparation of particles	Aerosol generated in glass atomiser
Species	Wistar rats
Quarantine	7 days on arrival at the test facility under standard laboratory conditions.
Age at exposure	10 weeks
Type of exposure	Head and nose exposure
Body weight at treatment	Males:180-208g
	Females : 150-163g
Diet	Rats/mice (pellet) Feed (Ad libitum)
Water	*Ad libitum*
	Deep bore-well water filtered through activated charcoal and exposed to UV rays
Housing	Individually housed in standard polypropylene rat cages with stainless steel top grill; steam sterilised clean paddy husk, changed twice during week 1 and 2 and thrice during week 3.
Temperature	21-25°C
Humidity	30-70%
Air changes	Air conditioned with adequate fresh air supply (12-15 air changes/hour)
Photoperiod	12 hour light and 12 hour dark cycle
Vehicule	Dimethyl sulphoxide (DMSO)
Concentration in vehicle	G1: DMSO 0% cypermethrin
	G2 : 60 % w/v cypermethrin in DMSO
Duration of exposure	4 hours
Controls	DMSO (G1)
Examinations	Observation of toxic signs and pre-terminal deaths were done immediately after exposure, while releasing the rats from the restainers. There after, the observations for toxic signs and pre-terminal deaths were done once daily during days 2 to 15. Body weight were recorded on day 1, 8 and 15. Rats surviving til the end of the observation period were sacrificied and subjected to detailes necropsy.
	Logit method (J. Berkson, J.Am.Stat. Ass. 39. 357-65, 1944)

Acute Inhalation Toxicity – Rat (1)

Rat, cypermethrin, Inhalation Exposure (LC50)

- ***Results and discussion***

Clinical signs	There were no toxic signs in G1 group. In G2 group, lethargy, slight/severe salivation, eye discharge, nasal discharge and tremors were observed in all rats on day 1, lethargy was observed in all rats on day2. All rats were normal from day 3 onwards. Theres were no pre-terminal deaths.
Pathology	No abnormality was detected at necropsy.
Other	Body weight: all rats gained body weight during observation period.
LD_{50}	LC50 > 3.56 mg/L
Conclusion	The acute inhalation (4 hours) LC_{50} value of cypermethrin technical is more than 3.56mg/l of chamber air.
Reliability	*1*
Deficiencies	No

Acute Inhalation Toxicity – Rat (2)

Rat, cypermethrin, Inhalation Exposure (LC50)

Reference	[redacted], (1985); Acute Aerosol Inhalation Toxicity in the Rat of CGA 55186 Tech. (cypermethrin); Ciba-Geigy Ltd, report No.:840047 (CYP/T82g), 2 May 1985 (unpublished) Dates of experimental work: 31 July 1984 – 5 December 1984
Guideline study	Existing study with protocol based on method B.2. of Directive 92/69/EEC (corresponding OECD guideline 403)
GLP	No; GLP was not compulsory at the time the study was performed

- ***Materials and methods***

Deviations	No
Test material	CGA 55186 tech (cypermethrin cis:trans/40:60)
Lot/Batch number	307046
Purity	92.6%
Concentrations	Nominal concentration : 0, 2217, 3119, 3478, 5170 mg/m³ Analytical concentration: 0, 970, 1926, 3462, 5328 mg/m³
Particle size	MMAD 1.6-2.9 µm, GSD 2.3-2.7, 82-94% of airborn particles had a diameter smaller than 7 µm.

Acute Inhalation Toxicity – Rat (1)

Rat, cypermethrin, Inhalation Exposure (LC50)

Type or preparation of particles	Aerosol generated in a pneumatic nebulizer
Type of exposure	Nose only
Vehicle	Ethanol
Concentration in vehicle	20 % w/w cypermethrin
Duration of exposure	4 hours
Controls	Ethanol (nominal concentration 32.4 g/m³)
Examinations	Mortality and clinical symptoms observed during exposure at 1, 2 and 4 hours, as well as 2 hours after exposure and then daily thereafter for 14 days. Dead animals removed twice daily on working days. Body weight recorded on days 7, 14 and at death. Gross necropsy at death and on all survivors at the end of the observation period.
Method of determination of LD_{50}	Logit method (*J. Berkson, J.Am.Stat. Ass. 39. 357-65*, 1944)
• ***Results and discussion***	
Clinical signs	In the control (ethanol) group, sedation, dyspnea, exophthalmos, and ruffled fur were observed at the day of application. In the test groups, dyspnea, ruffled fur, curved body position, and convulsions were observed for both sexes to a similar extent but with a dose-dependent increase in intensity and duration. In addition, the high dosed males showed extreme irritability and hyperkinetic behaviour. Surviving animals recovered within 9 days. All deaths occurred during the exposure period or within 2 hours thereafter.
Pathology	About half of the animals in the two higher dose groups showed mottled, hemorrhagic, or edematous lungs, as well as dilatation of the stomach. In 2 males and 1 female exposed to 3462 mg/m3, dilatations of the heart were found.
Other	Body weight: male rats showed significantly lower weight gain during the first week after exposure and compensated with increased gain in the second week. Females were not significantly affected.
LD_{50}	LC50 male : 3281 mg/m³ (= 291 mg/kg bw) LC50 female : 5038 mg/m³ (= 448 mg/kg bw) LC50 : 3894 mg/m³ (= 327 mg/kg bw)
• ***Conclusion***	A LC_{50} = 3281 mg/m³ is used for risk characterisation purpose.
Reliability	*2*
Deficiencies	No

In the Draft Renewal Assessment Report (DRAR) of Cypermethrin prepared in the context of the application for renewal of approval of the a.s. in Annex I to Councel Directive 91/414/EEC, a new study ([redacted], 2005) was discussed. This study was added because another vehicle is used: DMSO (ethanol in the [redacted]., 1985). Therefore, the study is also discussed in the CLH report of Cypermethrin cis: trans/40:60. Moreover this study is GLP, the only other reliable study ([redacted]., 1985) was performed before GLP was compulsory and has therefore only a reliability of 2. As this study was performed with cypermethrin (cis:trans/53:47) this study has to be seen as supplementary. [redacted]., (2005) tested the acute inhalative toxicity of cypermethrine technical (cypermethrin cis:trans/53:47) on Wistar rats on 5 males and 5 females per dose level, 10 weeks old, 180-208g (males) 150-163g (females). Animals were exposed to 0, and 3,56 mg/L analytical concentration of cypermethrine in DMSO vehicle for 4 hours. For group 1 (G1) dimethyl sulphoxide was used. For group 2 (G2), 120 g of the test substance was dissolved and made up to 200 mL with DMSO to get a concentration of 60% w/v because cypermethrin was too viscous to generate an aerosol (Table 21).

Exposure chambers were made of rectangular stainless steel/glass, pyramidal at top and bottom and a volume of 0.5 m^3. The rats were housed in special rat restrainers mode of cylindrical glass and brass metal, which had holes at anterior end and were closed at posterior end allowing only head and nose exposure. A glass atomiser was used.

Particle size (in µm) was measured with a particle size analyser and the % v/v of oxygen with an oxygen analyser. Sample extracts (mg/L of chamber air) were analysed with a Gas Chromatograph with PC-based data system.

Pre-study

In-house bred rats (2 males and 2 females/group) were exposed to aerosol generated under the following conditions:

Table 20: Test parameters for the pre-study, [redacted]., (2005)

	Pre-study I	Pre-study II
Atomiser used	Glass atomiser	Glass atomiser
Atomiser pressure	1.2 kg/cm^2	1.2 kg/cm^2
Injection rate	0.4 mL/min	0.8 mL/min
Airflow rate	28 L/min	28 L/min
Sampling duration	10 min	10 min
Sampling rate	10 L/min	10 L/min
Dose [% w/v]	20 (G1) and 60 (G2)	60 (G3)

Main study

For the main study, all the conditions were maintained similar to pre-study II. Injection rate was 0.8 mL/min and atomiser pressure of 1.2 kg/cm^2. Based on pre-study, the following doses were selected:

Table 21: Test parameters for the main study, [redacted]., (2005)

Group	Test item concentration	Injection rate (mL/min)	Sex	No. of rats

G1	Vehicle control (DMSO)	0.8	M F	5 5
G2	60% w/v in DMSO	0.8	M F	5 5

M: male; F: female; DMSO: dimethyl sulphoxide

Results

The mean aerosol particle size was 1.11 ± 0.67 µm for G1 and 1.23 ± 0.70 µm for G2. O_2 content were between 20.6 and 20.8 % (v/v). Analytical measurement of cypermethrin technical content were 0 mg/L for G1 and 3.56+/- 0.83mg/L for G2.

No animal died during the test period (15 days). All animals gained body weight during observations (Table 22). In G2 group, lethargy, slight/severe salivation, eye discharge, nasal discharge and tremors were observed in all rats on day 1, lethargy was observed in all rats on day 2. All rats were normal from day 3 onwards. At the end of the study, animal were sacrificed following exposure. At necropsy, no abnormality was detected.

The acute inhalation (4 hours) LC50 value of cypermethrin technical is more than 3.56 mg/L, which represents the maximum achievable concentration.

Table 22: Body weight of rats in [redacted], (2005) study

Group and dose (mg of test item/ L of air)	Sex	Body weight		
		Initial	8th day	15th day
223G2421 Vehicle Control Dimethyl Sulphoxide	M	180	218	250
	M	191	212	229
	M	185	198	227
	M	203	223	242
	M	183	200	222
	F	150	166	179
	F	153	157	181
	F	160	172	185
	F	159	166	173
	F	151	159	164
G2 3.56	M	208	223	248
	M	193	212	249
	M	192	205	230
	M	185	195	223
	M	186	207	230
	F	150	161	173
	F	160	171	182
	F	154	166	180
	F	163	175	180
	F	160	171	178

M: Male; F: Female

[redacted]., (1985) tested the acute inhalative toxicity of cypermethrin technical (cypermethrin cis:trans/40:60), in rat (Tif: RAI f (SPF), F3-crosses of RII 1/Tif x RII 2/Tif) on 5 males and 5 females per dose level, young adults (no more precision available), 229±29g (males) and 212±13g (females). Animals were exposed to 0, 2217, 3119, 3478, 5170 mg/m³ nominal concentration, respectively 0, 970, 1926, 3462, 5328 mg/m³ analytical concentrations in ethanol vehicle for 4 hours. The deteremination of the LC50 was made using the Logit method (*J. Berkson, J.Am.Stat. Ass. 39. 357-65*, 1944). No additional information is available on the statistics. An LC50 = 3281 mg/m³ (aerosol) was determined by nose-only exposure for 4 hours. In control group, clinical sign observed were sedation, dyspea, exophthalmos, and ruffled fur, observed at the day of application. In the test group, clinical signs observed during the observation period were dyspnea, ruffled fur, curved body position, and convulsions observed for both sexes to a similar extent but with a dose-dependent increase in intensity and duration. In addition, the high dosed males showed extreme irritability and hyperkinetic behaviour. Animals died during the 4h exposure or within 2 hours following exposure at concentration 1926 mg/m³ (1 female, after 6 h from begining), 3462 mg/m³ (2 males and 1 female, after 4 h from begining) and 5328 mg/m³ (2 males after 3 h from begining; 3 males, 1 female after 4 h from begining; 2 females after 6 hours from begining) (Table 23). Surviving animals recovered within 9 days. Male rats showed significantly lower weight gain during the first week after exposure, but this was compensated by increased gain in the second week (Table 24). Body weight was not affected in females. At necropsy (*at death and on all survivors at the end of the observation period.)*, about half of the animals in the two highest dose groups showed mottled, hemorrhagic, or oedematous lungs, as well as dilatation of the stomach. In 2 males and 1 female exposed to 3462 mg/m³, dilatations of the heart were found.

Table 23: Occurrence of mortalities in rat, [redacted], (1985)

Conc. [mg/m³]	Number of dead / number of investigated	Time of death (range)	Observations
0	0/10 (♂ 0/5;♀ 0/5)	-	Dyspnea, exophthalmus, ruffled fur, sedation. All animals recovered 1 day post-exposure.
970	0/10 (♂ 0/5;♀ 0/5)	-	Dyspnea, ruffled fur, curved body position. All animals recovered by day 5.
1926	1/10 (♂ 0/5;♀ 1/5)	Within 2 hours after exposure	Dyspnea, ruffled fur, curved body position. All animals recovered by day 7.
3462	3/10 (♂ 2/5;♀ 1/5)	During exposure	Dyspnea, ruffled fur, curved body position, convulsions. All animals recovered by day 9.
5328	8/10 (♂ 5/5;♀ 3/5)	During exposure and within 2 hours after exposure	Dyspnea, ruffled fur, curved body position, convulsions. All animals recovered by day 7.

Table 24: Mean weight gain, [redacted], (1985)

Day of observation period	Sex	Means ± Standard deviations (grams)				
		Exposure concentration mg/m³				
		Control	970 ± 21	1926 ± 93	3462 ± 231	5328 ± 368
0#	Male	252 ± 8	209 ± 10	205 ± 8	271 ± 9	210 ± 5
7		292 ± 9	236 ± 9*<	238 ± 14	289 ± 11*<	na
14		323 ± 12	278 ±12*>	279 ± 20*>	330 ± 15	na
0#	Female	226 ± 9	204 ± 5	207 ±9	222 ± 11	199 ± 7
7		233 ± 8	213 ± 8	218 ± 5*>	234 ± 9*>	201 ± 8
14		249 ± 14	231 ± 3	234 ± 6	239 ± 10	221 ± 6

immediately before exposure
* mean weight gain differs significantly (P<0.05) from control (> = gain increased, < = gain decreased)
na no surviving animals in dose group

In conclusion, it is possible that a certain interaction could occur with the solvent, although this should be minimal. It is noted that exophthalmos was not seen in the dosed animals. In addition, the clinical signs of the treated animals (not the controls) were, besides ruffled fur and dyspnea also curved body position, and also convulsions from 3.462 mg/L onwards (dose relevant for classification), in a dose-dependent way.

4.2.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as ACUTE TOX

Table 25: Relevant findings on Acute Tox studies

Toxicological result	CLP criteria
Key studies:	
oral study in rats: ([redacted]., 2005) **REL:1** Cypermethrin cis:trans/40:60 Dose: 300, 2000 mg/kg bw/d Vehicle: refined groundnut oil Species: Rat, Wistar, female, 3/group Results: LD_{50} **500mg/kg bw/d**: Clinical sign in the G2F group (2000 mg/kg bw), toxic signs observed were slight/severe salivation, tremors, lethargy, ataxia and perineum wet with urine. Lung congestion detected at necropsy The results lead to classification as ACUTE TOX, Cat. 4	Oral: 300< LD_{50}<2000 mg/Kg bw
oral study in rats: ([redacted]., 1984a) **REL:2**	Oral: 300< LD_{50}<2000 mg/Kg bw

Cypermethrin cis:trans/40:60 Dose: 300, 600, 1200, 2500, 5000 mg/kg bw/d Vehicle: arachis oil Species: Rat, Tif:RAIf (SPF), m/f, 5/sex/group Results: **LD50 in males 1732** (1027-2922) mg/kg bw LD50 in females 2150 (1342-4024) mg/kg bw LD50 in both sexes 1945 (1449-2676) mg/kg bw: Clinical sign: Dyspnoea, exophthalmus, ruffled fur and curved body position, diarrhoea and tremor. Tonic clonic convulsion was observed from 600 mg/kg bw onwards. At higher concentrations also salivation, sedation and lateral/ventral body positions were observed. The results lead to classification as ACUTE TOX_{oral}, Cat. 4	
oral study in rats: (*Cantalamassa F.*, 1993) **REL: 3** Cypermethrin cis:trans/37:63 Dose: Not specified Vehicle: corn oil Species: Rat, Wistar, male, 10/groupResult: LD50= 250mg/Kg bw The result lead to classification as ACUTE tox Cat 3	Oral: 50< LD_{50}<300 mg/Kg bw
4 h inhalation study in rats: ([redacted], 2005) Cypermethrin cis:trans/53:47 Vehicle: DMSO Dose: Analytical concentration 3.56 mg/L SVC= 1.1E-05 **REL: 1** The result leads to no classification as ACUTE $TOX_{inhal,}$ Cat 4 since no fatalities was observed for the max concentration obtained in the test.	Dust/mist: 1.0< LC_{50}<5.0
4 h inhalation study in rats: ([redacted], 1985) **REL: 2** (GLP not compulsory at the time) Cypermethrin cis:trans/40:60 Vehicle: ethanol Dose: Analytical concentration: 0, 970, 1926, 3462, 5328 mg/m³ *Results:* LC50 male : **3281 mg/m³** (= 291 mg/kg bw) LC50 female : 5038 mg/m³ (= 448 mg/kg bw) LC50 : 3894 mg/m³ (= 327 mg/kg bw)	Gases: 2500< LC50<20000 ppm

The results leads to classification as ACUTE $TOX_{inhal.}$, Cat. 4.	

Apart from the isomer ratio, the oral toxicity of cypermethrin varies with the type of vehicle used, species and strain. In general, as discussed in the assessment report prepared for Cypermethrin under Biocidal Product Regulation (BPR), aqueous suspensions were the least toxic and non-polar solutions the most toxic. Oral LD50 values vary from 250 mg/kg bw (in oil) to > 5000 mg/kg bw (aqueous solutions). The Wistar rat was found more sensitive than the Tif:RAIf (SPF) rat strain. The most relevant and reliable studies used as key studies in the assessment report prepared for Cypermethrin under Biocidal Product Regulation (BPR), were also discussed in the scope of the CLH report. As can be seen in table 25, is corn oil the oil vehicle with the highest potential potency. However, this study has only a reliability of 3 in contrast with the 2 keys studies.

4.2.3 Conclusions on classification and labelling acute toxicity findings relevant for classification as ACUTE TOX

Regarding the effects observed in the acute oral route studies ([redacted]., (2005) and [redacted]., (1894a)), a classification as ACUTE Tox Cat4 for oral exposure route is warranted. The LD_{50} = 250mg/Kg bw calculated in this *Cantalamessa*, (1993) study would lead to a classification in acute tox cat 3 for oral route exposure. However, the level of details is so low that a reliability of 3 was attributed to the study and the conclusion is challengeable by the result of the two key studies available. Therefore the study is not deemed of suffisent value to classify the substance cypemethrin cis:trans/40:60 as ACUTE Tox, cat 3. Therefore a classification as ACUTE Tox 4 is required.

As regards to the classification as ACUTE Tox inhalation, the earlier acute inhalation study of[redacted], (1985) performed with cypermethrin (cis:trans/40:60) in ethanol had fatalities at the highest dose and thus the calculated LC50 of 3.28 mg/L/4h resulted in H332 (Harmful if inhaled). The observed effect in the [redacted], (1985) study are dose dependent and therefore can not be attributed to the solvent used. The fact that no fatalities occurred in the [redacted], (2005) study, which was performed with cypermethrin (cis:trans/53:47) in DMSO are not enough to exclude the result of the [redacted] study from 1985 in wich fatalities can not be attributed to the solvent only. A classification as ACUTE Tox inhal Cat 4 is required.

Classification/Labelling of the active substance 'cypermethrin cis:trans/40:60' for acute toxicity according to the criteria in CLP-Regulation (EC) No 1272/2008:

Acute Tox. 4; H302: **Harmful if swallowed**.

Acute Tox. 4; H332: Harmful if inhaled.

4.3 Specific target organ toxicity – single exposure (STOT SE)

Not evaluated in this dossier.

4.4 Irritation

4.4.1 Skin irritation

Not evaluated in this dossier.

4.4.2 Eye irritation

Not evaluated in this dossier.

4.4.3 Respiratory tract irritation

Not Evaluated in this dossier.

4.5 Corrosivity

Not evaluated in this dossier.

4.6 Sensitisation

4.6.1 Skin sensititsation

Not evaluated in this dossier.

4.6.2 Respiratory sensitisation

Not evaluated in this dossier.

4.7 Repeated dose toxicity

The following information was extracted from the toxicology chapter of the assessment report prepared for cypermethrin under Biocidal Product Regulation (BPR), from the DAR made for cypermethrin under PPP Regulation, and from open literature publications.

The purpose of these studies is to evaluate the STOT RE classification of cypermethrin. These studies are repeated dose toxicity studies and are therefore presented in this chapter 4.7. However, the discussion leading to the classification are discussed in the chapter 4.8.

In the tables 26, 28, and 33 below, key studies are reported on a light grey background whereas supportive study are reported on a white background. More details (when available) are reported in

the description of study and in the discussion of the findings relevant for classification (see chapt. 4.8).

It has to be stated that no regulatory and reliable studies are available of which it is 100% clear that they are performed with cypermethrin cis:trans/40:60 as no studies were performed with the pure racemic compound. All available regulatory studies were performed with the technical cypermethrin product **WL43467** of which is known that the ratio cis:trans varies from 50:50 to 40:60. The used **WL43467** in the repeated dose toxicity studies was: [cis-trans isomers of (S,R) α-cyano-3-phenoxy-benzyl (1R, 1S, cis, trans)-2-dimethyl-3-(2,2-dichlorvinyl) cyclopropane carboxylate], no reported cis-trans ratio or 1:1 mixture, batch no 19, 21, 30, purity unknown or 98% or 98.5%. **WL43467** falls under the **CAS Number 52315-07-8, EC Number 257-842-9, Index Number 607-422-00-4**. However, these older studies (performed in 1976, 1977, 1978) do not contain a certificate of analysis of the test item showing the analytical results of the cis-trans isomer ratio. It was accepted that these studies could be used for the evaluation of the active substance cypermethrin 40:60 in the framework of Directive 98/8/CE (Biocide). Refering to chapter 1.2 Composition of the substance of this CLH report, the cypermethrin cis:trans isomer ratio is 40(±5):60(±5). In table 7 in chapter 1.2 Composition of the substance it is shown that the most common cis:trans ratios are cis (40% min, 48% max), trans (60% max, 52% min). As for the older studies no exact cis:trans ratio has been determined/is known, the studies were accepted as these studies were probably performed with cypermethrin with a cis:trans ratio at the extreme limit acceptable for cis:trans/40:60.

Table 26: Short-term repeated dose toxicity of cypermethrin

Route	Method Guideline	TS	Duration of study	Species Strain Sex No/group	Dose levels frequency of application	Results	LO(A)EL	NO(A)EL	Reference
Oral feed	Method B7 with deviations: limited inquiries, absence of raw data Rel=3 GLP= no	Cypermethrin (WL43467, cis:trans/1:1, batch no 19, unknow purity)	5 weeks	Rat Charles River m/f 6/ sex/ group	25, 100, 250, 750, 1500 ppm. 1.25, 5, 12.5, 37.5, 75 mg/Kg bw/d. Daily Control= none	No test substance-related mortalities. **25, 100, 250 and 750 ppm**: No test substance-related changes **1500 ppm**: Clinical signs: piloerection, nervousness, uncoordinated movements from 2 weeks onwards in 4/6 ♂ and 1/6 ♀ Bw gain, food intake, terminal bw: reduced for m&f (no details) Organ weight: ⓿ abs. and rel liver weight in ♀ (no details) Clinical chemistry: ⓿ hemoglobin and blood urea conc. in ♂; ⓿ plasma alkaline phosphatase in ♀ (no details) **Neurotoxicity**	1500 ppm 75 mg/Kg bw/d	750 ppm 37.5 mg/Kg bw/d	[redacted] (1976)

Oral feed	Method B7 with deviations: limited inquiries, absence of raw data Rel= 3 GLP= no	Cypermethrin (WL43467, cis:trans/1:1, batch no 19, unknow purity)	5 weeks	Dog Beagle m/f 3/sex/group	0, 15, 150, 1500 ppm. 0, 0.375, 3.75, 37.5 mg/Kg bw/d. Daily	No test substance-related mortalities. **0, 15, 150 ppm**: No test substance-related changes **1500 ppm**: Clinical signs: apprehension, diarrhoea, vomiting, licking and chewing of the paws, whole body tremors and stiff exaggerated hind leg gait, ataxia. 2 animals (1♂ and 1♀) convulsed during week 1 and 5. Bw gain, food intake, terminal bw: ↓ bw gain (no details) Organ weight: ⓿ rel. thyroid weight in ♂&♀ (no details) Clinical chemistry: ⓿ WBC and KCCT at week 5 in ♂; ⓿ blood urea levels, ↓ blood glucose levels at week 5 in ♀ (no details) **Neurotoxicity**	1500 ppm 37.5 mg/Kg bw/d	150 ppm 3.75 mg/Kg bw/d	[redacted] (1976)
Oral	No guideline Rel= 3	Cypermethrin (Suminotomo Chem.), unknown purity	3 weeks	Rat albino males	0, 31.5 mg/Kg bw/d vehicle: corn oil	Liver: cytoplasmatic hypertrophy with intracytoplasmatic droplets. Mitochondrial ATPase activity: inhibitory effect (70.8%) **Liver toxicity**	Not determined	Not determined	*el-Toukhy and Girgis*, (1993)
Oral	No guideline Rel=3	Cypermethrin (Novartis), >91%	5 days	Rat, Wistar males	0, 75 mg/Kg bw/d vehicle: corn oil	No mortalities and clinical signs. Hepatic and cerebral tissues: enhanced peroxidation, as indicated by increased TBARS levels (see table 27) Control: Cerebral: 1.88 ± 0.04 Hepatic : 2.13 ± 0.06 Cyp 75mg/Kg : Cerebral: 2.11 ± 0.05 ($p < 0.05$) Hepatic : 2.85 ± 0.04 ($P<0.05$)	Not determined	Not determined	*Giray et al*., (2001)

Dermal	Method B.9 with deviations: Performed on abraded skin, under occluded patch, Limited clinical description, bilirubine and creatinine not measured, coagulation parameters not examined Rel =3	Cypermethrin 53:47, 91.5%	3 weeks (15 days)	Rabbit, New Zealand White m/f 10/sex/group	0, 2, 20, 200 mg/Kg bw/d vehicle: PEG300 (6 hours/day)	**2 and 20 mg/Kg bw/d:** Local effects: slight to mild erythema, dose dependent, slight to moderate oedema, dose dependent **200 mg/Kg bw/d:** ↓significative food intake, bw gain, weight of gonads (no more details) Local effects: erythema and oedema Slight to severe erythema, Slight to severe oedema Focal liver necrosis	200 mg/Kg bw/d	20 mg/Kg bw/d	[redacted] (1981) in 91/414 DAR for cypermethrin made by the BE CA
i.p.	No guideline Rel=3	Cypermethrintechnical grade (cis:trans/49.9:50.1), purity 91%	7 days	Rat Wistar Males, n=7	0, 300 mg/Kg bw/d vehicle: Pluronic F-68 daily	No test substance-related mortalities. At 300mg/kg: Clinical signs: scratching, salivation, somnolence, ataxia, convulsion and hind limb paralysis noted at every time point, on a daily basis during 7 days. Bw: reduced at d 7. (314.39 ± 28.6 to 279.59 ± 15.5 g) Organ weight: ⓿ rel liver weight (20%) Clinical chemistry: ↓serum albumin 3.69 ± 0.1 g:dl (control) to 2.99 ± 0.4 g:dl AST and ALT variation with statistically significant increase on day 2 (see table 21). AST increase maintained up to day 6. Histology liver at 72 h post-exposure: hepatocytes with ovoid nucleus; intra-cytoplasmatic droplets; mitochondria: normal to dilated, small mitochondria with electron dense inclusions. Proliferation and swelling of smooth endoplasmic reticulum more evident on d5 and subsequently. **Neurotoxicity and liver toxicity**	Not determined	Not determined	*Aldana et al.*, (1998)

[redacted] studies (1976): Both studies report only the main findings for the sub-acute oral toxicity. No tables containing numeral values or statistics are available. In spite of the fact that both studies are not conform the principles of acceptability because the absence of raw data and the fact that the exact cis:trans ratio is not analysed, the studies confirm that dogs are more sensitive than rats to the

toxic effects of cypermethrin. Therefore, the short-term studies are discussed and can been seen as more than supplementary or supportive.

Repeated dose toxicity

Short-term repeated dose toxicity (oral) - Rat

Reference	[redacted]. (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).
Guideline study	No Existing study comparable to method B7 of Directive 92/69/EEC
GLP	No GLP was not compulsory at the time the study was performed
Deviations	Yes. Limited enquiries compared to a more modern study, no raw data included in report. Results were expressed as statistically significant but no further details of statistical methods detailed in the report.
Test material	WL 43467 (cypermethrin) 1:1 mixture of cis/trans isomers, batch no 19, unknown purity
• ***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.
Control	Yes 14 animals/sex were used in the control group.
• ***Results and discussion***	The study report only the main findings for the sub-acute oral toxicity. No table containing numerical values or statistical analysis is avaible. 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 but no effect where observed on kidneys, testes, spleen brain,and heart on both male and female (no more details). No other changes were seen that could be attributed to the feeding of cypermethrin. No compound related histopathological effects were found on 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.. No signs of pyrethroid intoxication were seen in either male or female rats fed cypermethrin at concentrations of 750, 250, 100, or 25 ppm.

Repeated dose toxicity

Short-term repeated dose toxicity (oral) - Rat

	No mortality following cypermethrin administration occurs during the study.
• **Conclusion**	
LO(A)EL	LO(A)EL:1500 ppm (75 mg/kg bw/d)
NO(A)EL	NO(A)EL= 750 ppm (37.5 mg/kg bw/d)
Reliability	3
Deficiencies	Yes. Deviations from official protocol – limited findings and no raw data were included in the brief summary report. 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.

Repeated dose toxicity

Short-term repeated dose toxicity (oral) - Dog

Reference	[redacted] (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)
Guideline study	No, existing study comparable to method B7 of Directive 92/69/EEC.
GLP	No, GLP was not compulsory at the time the study was performed
Test material	WL 43467 (cypermethrin) 1:1 mixture of cis/trans isomers, batch no 19, unknown purity
Deviations	Yes. Limited enquiries compared to a more modern study, no raw data included in report. Results were expressed as statistically significant but no further details of statistical methods detailed in the report.
• ***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.
Control	Yes
• ***Results and discussion***	The study report only the main findings for the sub-acute oral toxicity. No table containing numerical values or statistical analysis is avaible. The following result were reported: At 1500ppm (37.5 mg/Kg bw/d), body weight gain, food intake and terminal body weight: loss of appetite, the latter accounting for a significantly decreased body weight gain (no more details). Clinical signs: apprehension, diarrhoea and vomiting, licking and chewing of the paws, whole body tremors and stiff exaggerated hind leg gait, ataxia. Two animals (1♂ and 1♀) convulsed during week 1 and 5 respectively.

Repeated dose toxicity

Short-term repeated dose toxicity (oral) - Rat

	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 more details). 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.
• ***Conclusion***	
LO(A)EL	LO(A)EL: 1500 ppm (37.5mg/Kg bw/d)
NO(A)EL	NO(A)EL= 150 ppm (3.75 mg/kg bw/d)
Reliability	3
Deficiencies	Yes. Deviations from official protocol – limited findings and no raw data were included in the brief summary report. 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.

Additional/supportive data from literature supporting the classification as STOT RE

Repeated dose toxicity

Short-term repeated dose toxicity (oral) - Rats

Reference	*Aldana L González de Mejía E., Craigmill A., Tsutsumi V., Armendariz-Borunda J., Panduro A., Rincón A.R.*, (1998) Cypermethrin increases apo A-1 and apo B mRNA but not hyperlipidemia in rats. Toxicoligy Letters 95: 31-39.
Guideline study	No
GLP	No
• ***Materials and methods***	Cypermethrin (49.9:50.1/ cis: trans) 91% purity was administred at 300 mg/Kg/d by intraperitoneal injection (I.P.) for 7 days to male Wistar rats. At least 7 animals were used for each time point and three independent experiment where carried out. Total protein, albumin, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were measured spectrophotometrically in serum using commercial kits (Lakeside Laboratories, Boehringer Mannheim, Germany).
Controls	The control animals received (i.p.). a corresponding amount of the vehicle (Pluronic F-68 (20%)).
• ***Results and discussion***	Clinical signs: scratching, salivation, somnolence, ataxia, convulsion and hind limb paralysis noted at every time point, on a daily basis during 7 days.

Repeated dose toxicity

Short-term repeated dose toxicity (oral) - Rats

	No statistically significant changes were detected in total serum proteins during the 7 days of cypermethrin treatment (Table 27). In contrast, albumin decreased at day 4 of cypermethrin treatment from 3.6±0.1 g/dl (control) to 2.9±0.4 g/dl (day 4) (P<0.05) AST and ALT determinations presented just slight variations with a statistically significant increase on day 2 as shown in table 27. This increase was maintained up to day 6 only for AST.
• *Conclusion*	Clear hepatotoxicity was found at 300 mg/Kg bw/d (i.p., 7 days).
Reliability	3

Table 27: Serum total protein , albumin , AST and ALT values of rats treated with cypermethrin; *Aldana et al*, (1998)

Days of treatment	Total protein (g/dl)	Albumin (g/dl)	AST (IU/l)	ALT (IU/l)
0	6.5 ± 0.4	3.6 ± 0.1	33.0 ± 2.0	15.0 ± 2.0
1	6.8 ± 0.6	3.5 ± 0.3	36.0 ± 2.0	12.0 ± 2.0
2	6.7 ± 0.2	3.6 ± 0.2	56.0 ± 8.0*	23.0 ± 3.0*
3	6.7 ± 0.1	3.5 ± 0.2	52.0 ± 4.0*	24.0 ± 4.0*
4	6.8 ± 0.3	2.9 ± 0.4*	47.0 ± 7.0*	18.0 ± 3.0
5	7.2 ± 0.3	2.8 ± 0.2*	69.0 ± 9.0*	17.0 ± 3.0
6	6.5 ± 0.4	3.0 ± 0.4*	42.0 ± 7.0*	18.0 ± 5.0
7	6.8 ± 0.3	3.3 ± 0.2*	40.0 ± 8.0	14.0 ± 2.0

Biochemical tests were determined as indicated in Section 2. Values are mean ± S.D. of duplicate determinations from at least seven animals.
*Significantly different from day 0 (P < 0.05).

Repeated dose toxicity

Short-term repeated dose toxicity (oral) – Rats

Reference	*El-Toukhy M.A., Girgis R.S.* (1993) In vivo and in vitro studies on the effect of larvin and cypermethrin on adenosine triphosphatase activity of male rats. J. Environ. Sci. Health B 28: 599-619.
Guideline study	No
GLP	No
Materials and methods	Liver is removed from albino rat and assayed for total adenosine triphosphatase (ATPase) activity following administration with single and repeated doses of larvin and cypermethrin (3 week oral treatment with 31.5 mg/Kg bw/d).
Results and discussion	The data indicate that the total (Na^+, K^+; Mg^{2+}) dependent ATPase in the liver tissue is significantly (no more information) inhibited by single and repeated doses of both insecticides. This inhibition is more pronounced by the repeated dose of cypermethrin than that of larvin. The in vitro study revealed that the inhibition encountered by different concentration of both larvin and cypermethrin is of the irreversible non-competitive type. This data indicate that these insecticides can cause biochemical and histopathological changes in the liver ATPase activity which may inhibit several biochemical functions of ATPase system such as: the active

Repeated dose toxicity

Short-term repeated dose toxicity (oral) – Rats

	transport of metal ions, oxidative phosphorylation of liver cells and generally the muscle contraction.
Conclusion	Rat liver ATPase was inhibited after a 3 week oral treatment with 31.5 mg/Kg bw/d.
Reliability	3

Repeated dose toxicity

Short-term repeated dose toxicity (oral) - Rats

Reference	*Giray B., Gürbay A., Hincal F.* (2001) Cypermethrin-induced oxidative stress in rat brain and liver is prevented by Vitamin E or allopurinol. Toxicology Letters 118: 139-146.
Guideline study	No
GLP	No
• ***Materials and methods***	Male albino Wistar rats, weighing 180±20 g were obtained from Animal Care Unit of Hacettepe University, and were maintained on a12-h light:12-h dark cycle in plastic cages. They were provided with standard laboratory chow and allowed free access to food and water. Cypermethrin was administered in (1 ml) corn oil by oral (ig) route, and control animals received a corresponding amount of corn oil in an identical manner. The single dose cypermethrin group received 170 mg:kg cypermethrin (2:3 LD_{50}) and were decapitated 4 or 24 h later. Repeated dosing was carried out at 75 mg:kg per day dose (1:3 LD_{50}), for 5 days and animals were decapitated 24 h after the last dose of cypermetrin. As the second part of the experiment, two groups of animals were pretreated, either with vitamin E or allopurinol as described, below, along with appropriate vehicle controls. In a first group, rats were pretreated with a loading dose of 100 mg:kg per day, ig, vitamin E in corn oil for 3 days, and then received a single maintenance dose of 40 mg:kg on the 4th day. Fifteen minutes later, animals received 170 mg:kg, ig, cypermethrin (Techn Grade, 91% purity) and were decapitated four hours later. In a second group, rats received a single dose of 100 mg:kg, ip,allopurinol (in 1 ml distilled water, adjusted to pH 11 with 1 N NaOH), and 15 min later, 170 mg/kg,ig, cypermethrin was administered. Four hours later animals were decapitated. The concentration of thiobarbituric acid reactivesubstances (TBARS) in whole homogenates of liver or cerebral tissues were determined, as a measure of lipid peroxidation according to the procedure of Ohkawa et al. (1979). The data were analyzed by one-

Repeated dose toxicity

Short-term repeated dose toxicity (oral) - Rats

way ANOVA and the significant differences between the control and the treated groups were evaluated by Student's *t*-test. A *P* value of 0.05 was considered significant.

Controls Yes (see detail in the text above)

- ***Results and discussion*** There was no mortality in any groups at any point in time. Minor symptoms of neurotoxicity, such as abnormal gait and nervousness, were noted in only a small number of animals (3/12) treated with single high dose of cypermethrin.

  Single or repeated oral administration of cypermethrin to rats caused enhanced lipid peroxidation in hepatic and cerebral tissues of rats, as indicated by increased TBARS levels (Table 34). The increase was significant at all measurement points, except in cerebral tissue 24 h after a single high dose. The highest elevation of TBARS in the

  brain was noted 4 h after the single high dose. However, in hepatic tissue, much higher.

  changes were observed and the effect of the single high dose of cypermethrin was found to increase with time. The increase was up to near 4 times the control level for single dose at 24 h. Whereas the increase caused by repeated low dosing was lower

  than that caused by single high dosing at any point in time. The level of conjugated dienes was measured as a second indicator of lipid peroxidation in single dose cypermethrin treated rats and found to be significantly increased in both tissues, when measured 4 h after a single high dose of cypermethrin administration (Fig.1). The enhancement in liver was much higher (~60%) also.

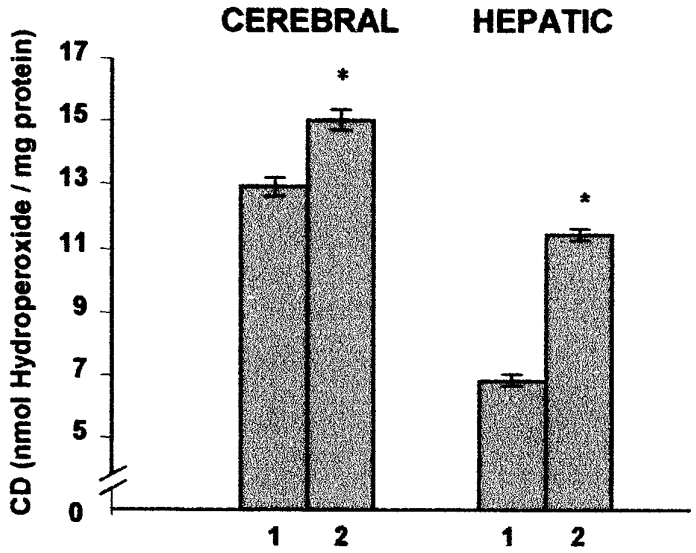


Fig. 1. Conjugated diene (CD) levels in cerebral and hepatic tissues of rats treated with a single dose of ig, cypermethrin. The treatment groups are indicated by: (1) Control groups (*n*_6), (2) Single dose cypermethrin, 170 mg:kg (*n*_6). Animals were decapitated 4 h after the cypermethrin dose or vehicle. Values are given as mean 9SEM. **P*B 0.5 versus control.

Repeated dose toxicity

Short-term repeated dose toxicity (oral) - Rats

• *Conclusion*	Oxidative stress induced by cypermethrin exposure (170mg/Kg bw) is shown in cerebral and hepatic tissues in rats (see table 27) by an elevation of the level of thiobarbituric acid reactive substances (TBARS) and conjugated dienes.
Reliability	3

Short term toxicity

15-day (3-week total) dermal toxicity (rat)

Reference	[redacted], (1981): Rabbits, dermal application of cypermethrin at 2, 20 or 200 mg/kg, 3 weeks
Guideline study	No Protocol not fully in compliance with the method B.9 of Directives 92/69/EEC (or 84/449/EEC).
GLP	No GLP was not compulsory at the time the study was performed
Deviations	Yes Limited enquiries compared to a more modern study, no raw data included in report. Results were expressed as statistically significant but no further details of statistical methods detailed in the report.
Test material	WL 43467 (cypermethrin) 1:1 mixture of cis/trans isomers
• ***Materials and methods***	Cypermethrin (91.5%; b.n° P19 ; cis:trans ratio : 53/47) was applied to the skin of 10 rabbits (New Zealand) /sex/dose over a period of 3 weeks ,5 times /week for 6 hour/day at a dose of 2, 20 or 200 mg/kg bw under occluded patch. The material was applied as a dilution in PEG 300. The skin of half of the animals was abraded.
• ***Results and discussion***	The liver necrosis observed at 200 mg/Kg bw did not show a clear zonal distribution within the lobules, although many involved the periportal zone. The small number of animals used makes it difficult to assess the significance of this finding. Inflammatory cell infiltration in the dermis was minimal or slight but occurred more frequently in test animals. Food intake, weight of gonads and body weight are significally (ANOVA or DUNNETT's test P> 0.05) reduced at 200mg/Kg bw.
• ***Conclusion***	
LO(A)EL	LO(A)EL= 200 mg/Kg bw
NO(A)EL	NO(A)EL= 20 mg/Kg bw
Reliability	3
Deficiencies	Yes. Limited findings and no raw data were included in the brief summary report. 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.

Subchronic Toxicity

Table 28: Subchronic toxicity of cypermethrin

Route	Method Guideline	TS	Dura-tion of study	Species Strain Sex No/group	Dose levels frequency of application	Results	LO(A)EL	NO(A)EL	Reference
Oral feed	Deviating OECD 408: Histopathology not performed on all organs. Target organs were not examined at all doses. GLP = No Rel =2	Cypermethrin, WL43467, cis:trans/not reported, batch no 21, 98.5%	90 days	Rat, CD m/f 12/sex/group Control: 24♂ 23♀	0, 25, 100, 400, 1600 ppm. 0, 1.25, 5, 20, 80 mg/Kg bw/d. Daily	**25, 100, 400 ppm:** General health and behaviour unaffected. **400 ppm:** ⓿ kidney weight in ♂ (5%), no histological change. **1600 ppm:** Clinical signs: Ataxia, hypersensitivity and abnormal gait during the first 5 weeks (9/12♂ ;7/12♀). Mortality: 1 ♂ died, 3 were killed. 2 of these rats showed axon breaks and vacuolation of myelin in the sciatic nerve. **Neurotoxicity** Males and females: ↓ BW (♂ 17%, ♀ 10% sign, P<0,01), ↓ food intake (♂, ♀; sign p<0,01 during 5 first week)), ↓ Hb (♂ 4%, ♀ 6%; sign p< 0,05), ⓿ urea (♂ 20%, ♀ 39%; sign P< 0,01), ⓿ kidney weight (♂ 7%; sign p<0,05, ♀ 14% sign, p< 0,01), Males: ↓KCCT (11% sign p< 0,01), ⓿ K^+ (13% sign p< 0,05) Females: ↓ RBC (6% sign p< 0,01), ⓿ AP (40% sign p<0,01), ⓿ liver weight (10% sign, P< 0,01), ⓿ spleen weight (17% sign, P<0,01)	80 mg/Kg bw/d	20 mg/Kg bw/d	[redacted], (1976)
Oral feed	Deviating OECD 408: Means: standard deviation not calculated. GLP = No Rel =3	Cypermethrin, WL43467, cis:trans/not reported, batch no 30, 98%	90 days	Dog, Beagle m/f 4/sex/group	0, 5, 50, 500, 1500 ppm. 0, 0.125, 1.25, 12.5, 37.5 mg/Kg bw/d vehicle: acetone Daily	**0, 5, 50, 500 ppm** No overt signs of intoxication and no other test compound related effects were found. **1500 ppm** Clinical signs: diarrhea, licking and chewing of the paws, whole body tremors, a stiff exaggerated hind leg gait, ataxia, incoordination and hyperaesthesia. These signs were observed along with ↓ food intake and ↓bw in both males and females (17-18%) Data not verifiable since sumary table not included in the full report!. Mortality: 2 ♂ and 2 ♀ were sacrificed during week 6 and 10, 10 and 12, respectively, for humane reasons. Haematology: ♀ ↓ RBC (6% sign p<0,05), ↓ KCCT (kaolin-cephalin clotting time) (21%, sign, p< 0,01). Pathology: focal bronchopneumonia in several animals. **Neurotoxicity**	37.5 mg/Kg bw/d	12.5 mg/Kg bw/d	[redacted] (1977)

Oral	No guideline GLP= No Rel =3	Cypermethrin, technical grade	90 days	Rat, albino Male, n = 35	0, 5, 10, 20, 40 mg/Kg bw/d. vehicle: ground nut oil daily	Dose-dependent decrease in delayed type hypersensitivity reaction on d 61 post-treatment; **20 mg/Kg bw/d** ⓿ adrenal weight (56%) stat sign p < 0.05 **40 mg/Kg bw/d** ↓spleen weight (25%), ⓿ adrenal weight (62%), leucopenia on d90 (↓35%) Stat sign p<0.05 **Immunotoxicity**	20 mg/Kg bw/d	10 mg/Kg bw/d	*Varshneya et al.* (1992)
Oral	No guideline GLP= No Rel = 3	Cypermethrin 25% EC	12 weeks	Rabbit New Zealand White Male, n=6	0, 24 mg/Kg bw every other day	↓ bw gain ⓿ rel. liver, spleen, kidney weight ⓿ plasma glucose, urea, creatinine, total bilirubine. ↓ plasma total protein, albumin. ⓿ plasma total lipid, cholesterol, TG, LDL, VLDL. ↓ HDL. ↓ Hb, RBC, PCV, ⓿ total leucocyte count. All results are statistically Significant (p<0.05)	24 mg/Kg bw every other day	< 24 mg/Kg bw every other day	*Yousef et al*. (2003a)

The 90 day oral toxicity of cypermethrin was studied in rats and dogs (regulatory studies).

Repeated dose toxicity

Subchronic Oral Toxicity - Rat

Reference	[redacted]. (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).
Guideline study	Existing study partly in compliance with method B.26 of Directive 87/302/EEC and OECD guideline 408 (1981).
Test material	WL 43467 (cypermethrin cis:trans/not reported; batch no 21; 98.5% purity)
GLP	No
Deviations	Histopathology not performed on all organs. Target organs not examined at all dose levels.
Statistics	Body and organ weights analysed by covariance. Chemical and haematological parameters examined using analysis of variance.

Repeated dose toxicity

Subchronic Oral Toxicity - Rat

• ***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).
Control	Yes
• ***Results and discussion***	The general health and behaviour of animals in dose groups up to 400 ppm were unaffected by ingestion of cypermethrin. The MTD was reached. In the 1600 ppm group many of the animals (9/12♂ ;7/12♀) showed hypersensitivity and abnormal gait during the first 5 weeks of the experiment. Of these group, 1 male died and 3 were killed for human 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.
• ***Conclusion***	
LO(A)EL	80mg/kg bw (1600ppm)
NO(A)EL	20 mg/kg bw (400 ppm)
Reliability	2
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 NO(A)EL 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.

Repeated dose toxicity

Subchronic Oral Toxicity - Dog

Reference	[redacted] (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).
Guideline study	The study is partly in compliance with method B.27 of Directive 87/302/EEC and OECD guideline 409 (1981).
GLP	No GLP was not compulsory at the time the study was performed.
Deviations	Means: standard deviations not given.
Purity	98.%
• ***Materials and methods***	4 Beagle dogs/sex/dose were fed a diet containing cypermethrin, WL43467(cypermethrin cis:trans/not reported) (Batch.n°.30; 98%) at concentrations of 0, 5, 50, 500 or 1500 ppm over a period of 13 weeks.

Repeated dose toxicity

Subchronic Oral Toxicity - Dog

	Converted dose : 0, 0.125, 1.25, 12.5, 37.5 mg/kg/d. Body and organ weights were analysed by covariance, using initial body weight as the covariate. Chemical and haematological parameters were examined using analysis of variance. The significance of any difference between treated and control was measured using Students's t test.
Control	Yes
• ***Results and discussion***	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. In the high dose group, 2 males and 2 females were sacrificed during week 6 and 10, 10 and 12 for human reasons. Clinical signs, were observed in the high dose group only along with reduced food consumption and bodyweight(17-18% cannot be verify due to lack of raw data for this parameter). In the top dose group, animals showed non-specific pathological changes, mainly in the lungs, where focal bronchopneumonia was seen in several animals (3♂; 1♀). Such changes can be expected in severe intoxication from any cause. 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***	
LO(A)EL	1500ppm = 37.5 mg/kg bw
NO(A)EL	500 ppm = 12.5 mg/kg bw
Reliability	3
Deficiencies	Yes. Protocol is 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.

Repeated dose toxicity

Subchronic Oral Toxicity - Rats

Reference	*Varshneya C., Singh T., Sharma L.D., Bahba H.S., Garg S.K.,* (1992) Immunotoxic responses of cypermethrin, a synthetic pyrethroid

	insecticide in rats. Short communication. Indian J. Physiol. Pharmacol. 36: 123*126.
Guideline study	No
GLP	No
Purity	Unknown
• ***Materials and methods***	Thirty five male albino rats of Wistar strain, weighing 150-190 g, were divided into 5 groups of 7 animals each. The animals of group nos. II, III, IV and V were administered technical grade cypermethrin in ground nut oil orally at the dose levels (mg/kg) of 5, 10, 20 and 40 once daily in the morning for 90 days. The insecticide solution was administered (ml/kg) as 0.5, 1**,** 2 and 4 percent solution to group nos. II, III, IV and V respectively by tuberculin syringe.
Control	The animals of Group I were administered isovolumetric amount of ground nut oil and served as experimental control.
• ***Results and discussion***	Significant ($p < 0,05$) leucopenic response at the level of 40 mg/kg on day 90 is observed. Delayed type hypersensitivity reaction, measured to evaluate cellular immune response, revealed a decrease by 24% and 27% in rats receiving cypermethrin at the dose levels of 20 and 40 mg/kg, respectively on day 61 post treatment. The decrease in cellular response was dose-dependent though the values were not significant statistically at $p < 0.05$ (no additional data available). On the other hand, no definite pattern was noticed in the humoral immune response as evidenced by mean serum haemagglutinin titres and haemolysin titres against sheep RBC on day 90 post treatment. The body weights of rats remained unchanged during the experimental studies. However, a significant decrease ($p < 0.05$) was observed in spleen weights at the highest dose level (25%). On the contrary to this, weights of adrenals had undergone a significant increase ($p < 0.05$) at the dose level of 20 mg/kg bw) (56%) and 40 mg/kg 62%). The weights of adrenals registered an increase in a dose dependent manner.
• ***Conclusion***	
LO(A)EL	LO(A)EL = 20 mg/Kg bw
NO(A)EL	NO(A)EL =10 mg/Kg bw
Reliability	3

Repeated dose toxicity

Subchronic Oral Toxicity - Rabbit

Reference	*Yousef M.I., El-Deerdash F.M., Kamel K.I., Al-Salhen K.S* (2003a) . Changes in some hematological and biochemical indices of rabbits induced by isoflavones and cypermethrin. Toxicology 189: 223-234.
Guideline study	No
GLP	No
Purity	25.%
• ***Materials and methods***	24 mature male rabbits were divided into four equal group of six rabbits each. One control group, the second groupe were given isoflavones (2mg/kg bw), the third groupe were given cypermethrin (24mg/kg bw) and the last group receive the combination of both respectively. Animals received the respective doses every other day for 12 weeks. Blood samples were collected every other week of the 12 weeks period and plasma was isolated and stored for further analysis. Collected blood sample were analysed for haemoglobin (hb) total erythrocyte count, (TEC); Packed cell volume (PCV) and total Leukocyte counts (TLC). Plasma sample were analyzed for total protein (TP) Albumin (A) concentrations Globulin (G) concentrations and A/G ratio was calculated. Plasma glucose level was measured and concentrations of urea, creatinine and total bilirubin was measured. Plasma was assayed for total lipids (TL), cholesterol, and triglycerides (TG). High density lipoprotein (HDL) and low density lipoprotein (LDL) were also determined. Very low density lipoprotein (VLDL) was calculated bydividing the values of TG by a factor of 5. Data were analyzed as a completely randomized design (*Steel and Torrie*,1980) using the General Linear Model procedure of SAS (1986). Means were compared by least significant difference (LSD) test (*Steel and Torrie*, 1980).
• ***Results and discussion***	The data indicated that plasma TL, cholesterol, TG, LDL and VLDL. were significantly ($p < 0.05$) increased by cypermethrin treatment, while HDL levels were decreased, (Table 29). Results indicated that treatment with cypermethrin caused significant increase ($p < 0.05$) in plasma glucose, urea, creatinine, and total bilirubin concentrations compared to control animals. Treatment with isoflavones alone had no significant effect on these parameters and counteracted or minimized the toxic effect of cypermethrin. Cypermethrin resulted in a significant ($p <0.05$) decrease in plasma total protein (TP) and albumin (A), while globulin concentration and A/G ratio were not affected (Table30). Cypermethrin treatment resulted in a significant ($p < 0.05$) decline in hemoglobin (Hb), total erythrocytic count (TEC) and packed cell volume (PCV), while total leucocyte count (TLC) increased (table 31). This increase in leukocyte counts may indicate to an activation of the animal's defense mechanism and immune system. There is a significant ($p < 0.05$) decrease in body weight gain, carcass weight and dressing percentage, and increase in the relative weights (g/100 g body weight) of liver, spleen and kidney of animals treated with cypermethrin compared with control rabbits (Table 32). On the other hand, cypermethrin did not cause any significant change in the relative weights of heart, lung, brain, and bladder compared with control. Treatment with isoflavones alone did not cause any significant

	change in all of these parameters, but alleviated the toxic effect of cypermethrin.
• *Conclusion*	Dose-dependent decrease was observed in delayed type hypersensitivity (DTH) reaction in rabbits (TLC increase) and induced moderate toxic effects on hemato-biochemical functions including hematological parameters and profiles of lipid, lipoproteins, protein, urea, creatinine, glucose and total bilirubin at 24 mg/Kg bw.
Reliability	3

Table 29: Plasma lipid and lipoprotein profiles during treatment of male rabbits with isoflavones (Iso.), cypermethrin (Cyp.) and their combination, *Yousef et al*, (2003a)

Lipids (mg/dl)	Experimental groups			
	Control	Isoflavones	Cypermethrin	Iso.+Cyp.
TL	623 ± 12.8^{b}	553 ± 12.9^{c}	756 ± 25.3^{a}	648 ± 13.2^{b}
TG	162.8 ± 2.3^{bc}	153.5 ± 2.5^{c}	178.0 ± 4.6^{a}	167.6 ± 3.2^{b}
Cholesterol	186.8 ± 1.8^{c}	172.4 ± 4.5^{d}	217.9 ± 3.7^{a}	196.3 ± 2.0^{b}
HDL	39.4 ± 0.30^{b}	43.2 ± 0.67^{a}	35.5 ± 0.48^{c}	38.9 ± 0.22^{b}
LDL	115.4 ± 2.1^{b}	98.9 ± 5.1^{c}	144.6 ± 3.3^{a}	124.7 ± 2.2^{b}
VLDL	32.5 ± 0.46^{bc}	30.7 ± 0.50^{c}	35.5 ± 0.93^{a}	33.5 ± 0.65^{b}

Values are expressed as overall means $\pm$SE; $n = 6$ for each treatment group. ^{abc} Means values within a row not sharing a common superscript letter were significantly different, $P < 0.05$. HDL: high-density lipoprotein. TL: Total lipids; TG: Triglycerides; LDL: Low desity lipoprotein; VLDL: Very low density lipoproteins.

Table 30: Plasma glucose, urea, creatinine, total bililrubin, total protein (TP), Albumin (A) and globulin (G) concentrations during treatment of male rabbits with isoflavones (Iso), cypermethrin (Cyp) and their combination, *Yousef et al*, (2003a)

Parameter	Experimental groups			
	Control	Isoflavones	Cypermethrin	Iso.+Cyp.
Glucose (mg/dl)	100.7 ± 0.90^{c}	100.1 ± 0.95^{c}	110.7 ± 1.8^{a}	105.6 ± 1.2^{b}
Urea (mg/dl)	28.3 ± 0.59^{b}	27.9 ± 0.63^{b}	31.7 ± 0.61^{a}	29.3 ± 0.50^{b}
Creatinine (mg/dl)	0.73 ± 0.02^{b}	0.72 ± 0.02^{b}	0.83 ± 0.03^{a}	0.76 ± 0.02^{b}
Bilirubin (mg/dl)	1.24 ± 0.03^{b}	1.22 ± 0.03^{b}	1.39 ± 0.05^{a}	1.28 ± 0.04^{ab}
TP (g/dl)	8.56 ± 0.12^{a}	8.61 ± 0.12^{a}	7.80 ± 0.13^{c}	8.23 ± 0.17^{b}
A (g/dl)	5.91 ± 0.17^{a}	5.96 ± 0.16^{a}	5.24 ± 0.13^{b}	5.61 ± 0.13^{ab}
G (g/dl)	2.65 ± 0.13^{a}	2.68 ± 0.11^{a}	2.55 ± 0.12^{a}	2.61 ± 0.14^{a}

Values are expressed as overall means $\pm$SE; $n = 6$ for each treatment group. ^{abc} Means values within a row not sharing a common superscript letter were significantly different, $P < 0.05$.

Table 31: Blood haemoglobin (Hb), total erythrocyte count (TEC), Packed cell volume (PVC) and total leukocyte count (TLC) during treatment of male rabbits with isoflavones (Iso.) Cypermthrin (Cyp.) and their combination

Parameter	Experimental groups			
	Control	Isoflavones	Cypermethrin	Iso.+Cyp.
Hb (g/dl)	11.9 ± 0.17^{ab}	12.4 ± 0.15^{a}	11.2 ± 0.24^{c}	11.7 ± 0.15^{b}
TEC ($\times 10^6$)	5.7 ± 0.08^{a}	5.6 ± 0.12^{ab}	5.0 ± 0.12^{c}	5.3 ± 0.09^{b}
PCV (%)	39.1 ± 0.19^{a}	40.4 ± 0.15^{a}	37.0 ± 0.48^{b}	38.4 ± 0.41^{a}
TLC ($\times 10^3$)	7.9 ± 0.19^{b}	7.7 ± 0.23^{b}	9.5 ± 0.30^{a}	8.2 ± 0.27^{b}

Values are expressed as overall means $\pm$SE; $n = 6$ for each treatment group. ^{abc} Means values within a row not sharing a common superscript letter were significantly different, $P < 0.05$.

Table 32: Effect of isoflavones (Iso.) cypermethrine (Cyp.) and their combination on relative organ weights (g/100g body weight) and slaughter performance of male rabbits

Parameter	Experimental groups			
	Control	Isoflavones	Cypermethrin	Iso.+Cyp.
Liver	2.23 ± 0.13^{b}	2.10 ± 0.17^{b}	2.69 ± 0.20^{a}	1.93 ± 0.06^{c}
Kidney	0.46 ± 0.01^{b}	0.44 ± 0.01^{b}	0.50 ± 0.01^{a}	0.44 ± 0.01^{b}
Lung	0.35 ± 0.02^{a}	0.35 ± 0.01^{a}	0.38 ± 0.01^{a}	0.35 ± 0.005^{a}
Brain	0.34 ± 0.03^{a}	0.34 ± 0.007^{a}	0.35 ± 0.02^{a}	0.33 ± 0.01^{a}
Spleen	0.044 ± 0.003^{b}	0.041 ± 0.002^{b}	0.064 ± 0.004^{a}	0.043 ± 0.006^{b}
Bladder	0.12 ± 0.011^{a}	0.11 ± 0.009^{a}	0.11 ± 0.009^{a}	0.12 ± 0.002^{a}
Heart	0.23 ± 0.004^{a}	0.22 ± 0.004^{a}	0.23 ± 0.010^{a}	0.21 ± 0.002^{a}
Carcass weight (g)*	1763 ± 44.7^{a}	1666 ± 17.7^{a}	1327 ± 73.9^{b}	1627 ± 55.1^{a}
Dressing (%)**	56.0 ± 0.66^{ab}	60.0 ± 0.71^{a}	52.6 ± 3.41^{b}	58.0 ± 1.42^{ab}

Note: Values are expressed as means $\pm$SE; $n = 3$ for each treatment group. ^{abc} Means values within a row not sharing a common superscript letter were significantly different, $P < 0.05$.

* Carcass weight is the weight of killed animals after removing of head, legs and offals (lungs, heart, liver and kidneys).

** Dressing (%) = (carcass weight/live body weight) $\times$ 100

Table 33: Chronic toxicity of cypermethrin

Route	Method Guideline	TS	Dura-tion of study	Species Strain Sex no/group	Dose levels frequency of application	Results	LO(A)EL	NO(A)EL	Reference
Oral feed	Deviating OECD 453 Deviations: low number of rats; blood albumin, glucose, glucose, GGT, ornithine decarboxylase not measured; urinalysis not performed. GLP= No Rel= 2	Cypeermethrin, WL43467, cis:trans/1:1, batch no 21, 98%	24 months	Rat: Wistar m/f 24/sex/group	0, 1, 10, 100, 1000 ppm. 0, 0.05, 0.5, 5, 50 mg/Kg bw/d Daily	No test substance related mortalities or signs of clinical toxicity in any of the treatment groups. Histopathology sciatic nerves: at 1 year and later sciatic nerves showed very small numbers of nerve fibers exhibiting the changes of Wallerian degeneration. Lesions consisted of swelling and fragmentation of axons and myelin. There was no difference in severity between dose groups. **1000 ppm:** Food consumption: ↓ (♂ 7%, ♀ 10%) Body weight: ↓ (♂ 7%, ♀ 7%) Hematology: platelets ❶ (♀ 4%) Clinical chemistry:liver PNOD ❶ (♂,♀), urea ❶ (♂ 58%), AP ↓ (♂ 33%) Other (minor) changes in hematological and clinical chemical parameters were not considered of toxicological significance as not supported by histopathological or other evidence of tissue damage. Organ weights: significant changes: Testes, rel.:10% ↓ (♂, 6 months) Liver, abs, rel:12%, 18% ❶ (♂, 18 mth) Heart, rel: 6%❶ (♂, 6 mth) Heart, abs:28% ↓ (♂, 12 mth) Kidney, rel: 8%❶ (♂, 12 mth; 9%❶♀, 6 mth) Kidney, abs:7%❶ (♂, 18 mth)	1000 ppm 50 mg/Kg bw/d	100 ppm 5 mg/Kg bw/d	[redacted] (1978)

Chronic Toxicity / Carcinogenicity

Rat-combined chronic toxicity/carcinogenicity study

Reference [redacted] (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).

Guideline study No Existing study. Method used comparable to method B.30 of Directive 87/302/EEC (corresponding OECD guideline 453).

Chronic Toxicity / Carcinogenicity

Rat-combined chronic toxicity/carcinogenicity study

GLP	No GLP was not compulsory at the time the study was performed.
• ***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.
Control	Yes (48animals/sex)
• **Results and discussion**	Test substance related effects seen at the high dose level (50 mg/Kg bw/d) including reduced food consumption and reduced body weight in males and femalescomparaid to controls, but no substance related mortality was observed (table 35). Minor statistically significant fluctuations seen in haematological parameters in the interim and 2 years groups, these lacking any compound related trends and therefore not considered to be of toxicological significance. Increased activity of p-nitroanisole-O-demethylase activity (liver PNOD) observed in both males and females confirmed that cypermethrin is a weak CYP II B1 inducer, AP activity was reduced. Most changes seen in absolute and relative organ weight did not show consistent patterns and were not correlated with histopathological or clinical chemistry changes. Increased liver weight associated with enzyme activity induction and increased kidney weight associated with the increase in blood urea. Histopathology: at 1 year and later sciatic nerves showed very small numbers of nerve fibers exhibiting the changes of Wallerian degeneration. Lesions consisted of swelling and fragmentation of axons and myelin. No difference in the intensity of the lesions in the proximal sciatic nerve and the distal. The appearances in the sciatic nerves were observed with the same incidence at all doses including the concurrent control animals. Statistical analysis revealed no evidence of increased risk of tumour development over the 2 years period The general health and behaviour of control and treated rats were similar throughout the study. Clinical signs were not compound related.
• **Conclusion**	Cypermethrin is not carcinogenic in this study. NO(A)EL = 100 ppm = 5 mg/kg bw/day
Reliability	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.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

It has to be stated that no regulatory and reliable studies are available of which it is 100% clear that they are performed with cypermethrin cis:trans/40:60 as no studies were performed with the pure racemic compound. All available regulatory studies were performed with the technical cypermethrin product **WL43467** of which is known that the ratio cis:trans varies from 50:50 to 40:60. The used **WL43467** in the repeated dose toxicity studies was: [cis-trans isomers of (S,R) α-cyano-3-phenoxy-benzyl (1R, 1S, cis, trans)-2-dimethyl-3-(2,2-dichlorvinyl) cyclopropane carboxylate], no reported cis-trans ratio or 1:1 mixture, batch no 19, 21, 30, purity unknown or 98% or 98.5%. **WL43467** falls under the **CAS Number 52315-07-8, EC Number 257-842-9, Index Number 607-422-00-4**. However, these older studies (performed in 1976, 1977, 1978) do not contain a certificate of analysis of the test item showing the analytical results of the cis-trans isomer ratio. It was accepted that these studies could be used for the evaluation of the active substance cypermethrin 40:60 in the framework of Directive 98/8/CE (Biocide). Refering to chapter 1.2 Composition of the substance of this CLH report, the cypermethrin cis:trans isomer ratio is 40(±5):60(±5). In table 7 in chapter 1.2 Composition of the substance it is shown that the most common cis:trans ratios are cis (40% min, 48% max), trans (60% max, 52% min). As for the older studies no exact cis:trans ratio has been determined/is known, the studies were accepted as these studies were probably performed with cypermethrin with a cis:trans ratio at the extreme limit acceptable for cis:trans/40:60.

Furthermore, except for 1 dermal repeated dose study, only repeated dose studies performed by oral administration are available and can be taken in consideration.

Summary of short term repeated dose toxicity studies.

The 5-week short term oral toxicity of cypermethrin was investigated in rats ([redacted]., 1976). In rats, a dose of 75 mg/Kg bw/d resulted in clinical signs of toxicity including piloerection, nervousness, uncoordinated movements from week 2 onwards. No mortality occurred. Food consumption and body weight gain and terminal body weight were significantly reduced in both males and females (no more precision). In females, a significant increase (no more precision) in relative liver weight was observed and an increase in plasma alkaline phosphatase activity. In males, haemoglobin and blood urea concentrations were significantly increased (no more precision). The NO(A)EL (oral, rat) was established at 37.5 mg/Kg bw/d for a 5 weeks exposure.

In the same study, [redacted]., (1976), the toxicity of cypermethrin was investigated on dogs. Compaired with the rats, the dog was more sensitive species to cypermethrin toxicity. In dogs, clinical signs were already observed at a dose of 37.5 mg/Kg bw/d, characterized by apprehension, diarrhoea, vomiting, licking and chewing of the paws, whole body tremors and stiff exaggerated hind leg gait, and ataxia. The number of animal concerned by this symptoms is no detailed in the study. Two animals (1 female, 1 male) convulsed during week 1 and 5 respectively but no mortality occurred. Body weight gain was reduced due to the observed loss of appetite in both males and females. In females, blood urea concentrations were increased and blood glucose levels decreased at week 5. In males, WBC and KCCT values were significantly (no more info) increased at week 5.

Relative thyroid weight was increased in both males and females. NO(A)EL oral, dog, 5 weeks = 3.75 mg/Kg bw/d.

The [redacted] study (1976) reports only limited main findings for the sub-acute oral toxicity. No raw data, no tables containing numeral values or statistics are included in the brief summary report. Results are only given in a short descriptive way. No more data is available than already incorporated under chapter 4.7 or here under chapter 4.8. In spite of the fact that both studies are not conform the principles of acceptability because the absence of raw data and the fact that the exact cis:trans ratio is not analysed, the studies confirm that dogs are more sensitive than rats to the toxic effects of cypermethrin. Therefore, the short-term studies are discussed and can been seen as more than supplementary or supportive.

Additional information from literature studies:

Neurotoxicity

Neurotoxicity was also demonstrated in the open literature.In Aldana et al. (1998) neurotoxicity was demonstrated at 300 mg/kg bw/d (cypermethrin cis:trans 49.9:50.1, 91% purity, i.p., 7 days, only 1 dose tested). Clinical signs: scratching, salivation, somnolence, ataxia, convulsion and hind limb paralysis noted at every time point, on a daily basis during 7 days. However, no more detailed information is available.

Hepatotoxicity

Apart from the clear neurotoxicity observed in the studies presented above, open literature also demonstrates hepatotoxicity. Within those, clear hepatotoxicity was found at 300 mg/Kg bw/d (i.p., 7 days) (*Aldana et al.* 1998) with an increase in AST level in male Wistar rats. In another study, effects on rat ATPase was observed by *El touky and Girgis*, (1993).

Rat liver ATPase was inhibited after a 3 week oral treatment with 31.5 mg/Kg bw/d (*El-Toukhy and Girgis*, 1993).

Oxidative stress induced by cypermethrin exposure is shown in cerebral and hepatic tissues in rats (see below) by an elevation of the level of thiobarbituric acid reactive substances (TBARS) and conjugated dienes (*Giray et al.* 2001).

Table 34: *Giray et al.* (2001): TBARS levels measured in cerebral and hepatic tissues in rats treated with cypermethrin

Tissue	Control group ($n = 20$)[b]	Cypermethrin treated group				
		Cypermethrin 75 mg/kg per day for 5 days ($n = 5$)[c]	Cypermethrin (24 h) 170 mg/kg ($n = 5$)[c]	Cypermethrin (4 h) 170 mg/kg ($n = 6$)[d]	Cypermethrin (4 h) + Allopurinol ($n = 6$)[e]	Cypermethrin (4 h) + Vitamin E ($n = 6$)[f]
Cerebral	1.88 ± 0.04	2.11 ± 0.05**	1.85 ± 0.08	2.55 ± 0.05*	1.75 ± 0.05**	1.76 ± 0.05**
Hepatic	2.13 ± 0.06	2.85 ± 0.04*	8.22 ± 1.00*	3.42 ± 0.33*	2.23 ± 0.04**	1.98 ± 0.06**

[a] Values are given as nmol TBARS/mg protein (mean ± SEM).

[b] Three groups ($n = 4$) of vehicle (corn oil) control and two groups ($n = 4$) of pretreatment plus vehicle control experiments were performed parallel to each treatment group. Mean value of all the groups was used as the overall control value, since there were no significant differences among groups.

[c] Animals were decapitated 24 h after the last dose of cypermethrin.

[d] Animals were decapitated 4 h after the cypermethrin dose.

[e] Animals received 100 mg/kg, ip, allopurinol and 15 min later 170 mg/kg cypermethrin, and were decapitated 4 h later.

[f] Animals were pretreated with 100 mg/kg per day, ig, Vitamin E for 3 days and with a dose of 40 mg/kg on the 4th day. Fifteen minutes later, they received 170 mg/kg cypermethrin and were decapitated 4 h later.

* $P < 0.05$ versus control group.

** $P < 0.05$ versus single dose cypermethrin (4 h).

Rabbits: Administration of cypermethrin in a 3-week (15-day exposure) dermal toxicity study ([redacted], 1981) in rabbits on abraded skin under occluded patch resulted in irritation of the skin which was associated to systemic effects such as focal liver necrosis. The severity of local effects (erythema and oedema) was dose-dependent. The liver necrosis observed at 200 mg/Kg bw did not show a clear zonal distribution within the lobules, although many involved the periportal zone.

Inflammatory cell infiltration in the dermis was minimal or slight but occurred more frequently in test animals. NO(A)EL dermal, rabbit, 3 weeks = 20 mg/Kg bw/d (91/414 DAR for cypermethrin). However, limited findings and no raw data were included in the brief summary report. 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.

Subchronic toxicity

Rats were fed a diet containing cypermethrin at concentrations of 0, 1.25, 5, 20, 80 mg/Kg bw/d over a period of 13 weeks ([redacted], 1976). MTD was reached. Daily observations were made on the general health and behavior of all the animals. Unfortunately, in this old study no raw data of the clinical signs were made available, nor was this data reported in a table. The only clinical observations data available is given in a descriptive way. Histopathology was not performed on all organs. Target organs were not examend at all doses. As in the 5 week studies, the target tissue/organ was the nervous system and liver. In the 80 mg/Kg bw/d (1600ppm) group rats showed hypersensitivity to sound and touch and abnormal gait (ataxia with splayed hind limbs) during the first 5 weeks of the experiment (9/12♂ ;7/12♀). Of this group, 1 male died and 3 males were killed. There is no more information available concerning the time of appearance and disappearance of the effects, intensity and severity of the reported neurotoxic effects. Two of these rats showed axon breaks and vacuolation of myelin in the sciatic nerve. No further sciatic nerve lesions were found in any of the other rats, even in the rats which had earlier been clinically affected. Clinical recovery was observed after the end of the 5th week and the food intake increased to a normal level. Body weight gain was statistically significantly reduced throughout the experiment (17%♂; 10%♀, sign, $p < 0,01$)

An increase in kidney (7% ♂/ 14% ♀, sign, $p < 0,01$), liver (10% ♀, sign, $p < 0,01$), and spleen (17% ♀,sign, $p < 0,01$) weight was observed at 80 mg/kg bw and at 20 mg/kg bw in kidney (5% ♂,sign, $p < 0,05$). Both in males and females, plasma urea was increased (20% ♂; 39% ♀, sign, $p < 0,01$) and haemoglobin was decreased (4% ♂,sign, $p < 0,05$; 6% ♀, sign, $p < 0,01$). In addition, in males kaolin-cephalin clotting time (KCCT) was decreased and Kalium was increased. In females RBC count was decreased, alkaline phosphatase was increased (11%). The health and behaviour of animals in the dose group up to 20 mg/Kg bw/d were unaffected by ingestion of cypermethrin.

Table 35: Summary findings for the 90-days oral toxicity study of cypermethrin in rats

Endpoint/dose	0		25 ppm		100 ppm		400 ppm		1600 ppm	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Mortality									**3**	
Clinical signs									**ataxia, splayed hind limbs, hypersensitivity**	
Body weight									**↓ 17%**	**↓ 10%**
Food intake									↓	↓
Haematology										
Hb									↓ 4%	↓ 6%
PCV										↓ 7%
RBC										↓ 6%
KCCT									**↓ 11%**	
Clinical chemistry										
Proteins										↓ 6%
K+									**↑ 13%**	
AP										**↑ 40%**
Urea									**↑ 20%****	**↑ 39%****
Organ weight (relative to terminal b.w.)										
Liver										**↑ 10%**
Kidney							**↑ 5%***	-	**↑ 7%****	**↑ 14%****
Spleen										**↑ 17%**

↑↓: statistically significant modifications; significance *p<0.05 or **p<0.01 added by RMS during revisal for certain endpoints

Dogs were fed a diet containing cypermethrin at concentrations of 0, 0.125, 1.25, 12.5, 37.5 mg/Kg bw/d over a period of 13 weeks ([redacted], 1977). The general health of all dogs was observed daily. Unfortunately, in this old study no raw data of the clinical signs were made available, nor was this data reported in a table. The only clinical observations data available is given in a descriptive way. Histopathology was not performed on all organs. Target organs were not examend at all doses. The feeding of 37.5 mg/Kg bw/d caused diarrhea, licking and chewing of the paws, whole body tremors, a stiff exaggerated hind leg gait, ataxia, incoordination and hypereasthesia in all except one animal in this dose group. Two of 4 males and 2 of 4 females were killed during the experiment because of the severe clinical signs of toxicity. The peculiar incoordinate gait of these dogs caused them to fall on many occasions and in two male dogs, the resulting superficial injuries made it necessary to kill them for human reason. The clinical condition of two female dogs deteriorated to such an extent that they too were killed on human grounds. There is no more information available concerning the time of appearance and disappearance of the effects, intensity and severity of the reported neurotoxic effects. Food intake and body weight gain were reduced (lost of 17-18% which cannot be verify due to lack of raw data in the original study) though to be due to the inappetence oberved in intoxicated dogs at the 1500 ppm dose level. RBC count and KCCT (kaolin-cephalin clotting time) were decreased (6%, sign, $p < 0,05$ and 21%, sign, $p < 0,01$ respectively) in the female dogs. Non-specific pathological changes, mainly in the lung (focal bronchopneumonia) were found in the animals of this group (3/4 males; 1/4 female), but no abnormalities were found in the central or peripheral nervous system. No overt toxicity (clinical signs, haematological and clinical chemistry parameters, pathological effects) was observed in the dose groups up to 12.5 mg/Kg bw/d.

In conclusion, at the highest dose levels tested (rat: 80 mg/Kg bw/d, dog: 37.5 mg/Kg bw/d) cypermethrin was found neurotoxic evidenced by clinical observations. In rats, neurotoxicity was confirmed by histopathology by peripheral nerve damage.

Table 36: Summary findings for the 90-days oral toxicity study of cypermethrin in dogs

Endpoint/dose	**0**		**5 ppm 0.125 mg/kg bw**		**50 ppm 1.25 mg/kg bw**		**500 ppm 12.5 mg/kg bw**		**1500 ppm 37.5 mg/kg bw**	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Mortality									2 (sacrificed) 1 at week 6 1 at week 10	2 (sacrificed) 1 at week 10 1 at week 12
Clinical signs									Diarrhoea, anorexian licking and chewing paws, whole body tremors, stiff exaggerated gait, ataxia, incoordination, hyperaesthesia	
Body weight									↘ 17%	↘ 18%
Food intake									↘	↘
Hematology										
RBC								↘ 6%*		↘ 6%*
KCCT								↘ 15%*		↘ 21%**
Histopathology										
Lung									Focal broncho pneumonia 3/4	Focal broncho pneumonia 1/4

This finding was also confirmed in more recent subchronic open literature studies (rats: *Varshneya et al.*, 1992; rabbits: *Yousef et al.*, 2003a).

In a short communication, *Varshneya et al.* (1992) report the immunotoxic effect of cypermethrin administration (0, 5, 10, 20, 40 mg/Kg bw in ground nut oil) orally for 90 days in the rat. Cypermethrin produced a significant ($p < 0,05$) leucopenic response at the level of 40 mg/kg on day 90 is observed. Delayed type hypersensitivity reaction, measured to evaluate cellular immune response, revealed a decrease by 24% and 27% in rats receiving cypermethrin at the dose levels of 20 and 40 mg/kg, respectively on day 61 post treatment. The decrease in cellular response was dose dependent though the values were not significant statistically at $p < 0.05$ (no additional data available). On the other hand, no definite pattern was noticed in the humoral immune response as evidenced by mean serum haemagglutinin titres and haemolysin titres against sheep RBC on day 90 post treatment. The body weights of rats remained unchanged during the experimental studies. However, a significant decrease ($p < 0.05$) was observed in spleen weight at the highest dose level (25%). On the contrary to this, weight of adrenals had undergone a significant increase ($p < 0.05$) at the dose level of 20 mg/kg bw

(56%) and 40 mg/kg (62%). The weights of adrenals registered an increase in a dose dependent manner. No clinical signs (neurotoxicity) were observed or described in this short communication.

The *Yousef et al.* (2003a) study reported that cypermethrin induced a dose-dependent decrease in delayed type hypersensitivity (DTH) reaction in rabbits (TLC increase) and induced moderate toxic effects on hemato-biochemical functions including hematological parameters and profiles of lipid, lipoproteins, protein, urea, creatinine, glucose and total bilirubin (increase, sign, $p < 0,05$) of rabbits when administrated orally 24 mg/Kg bw every other day for 12 weeks. No clinical signs (neurotoxicity) were observed or described in this study.

Chronic toxicity of cypermethrin was evaluated in rodent and relevant information can be extracted from non-rodent subchronic studies.

Rodent

Considering the chronic toxicity of cypermethrin, in a 24 month study with rats, [redacted] (1978); administered cypermethrin in the feed at doses of 0, 0.05, 0.5, 5.0, and 50 mg/Kg bw/d, respectively 0, 1, 10, 100, 1000ppm. Test substance related effects were seen at the high dose level (50 mg/Kg bw/d) including reduced food consumption and reduced body weight in males and females, but no substance related mortality (Table 29). Some minor statistically significant fluctuations were seen in haematological parameters in the interim and 2 years groups, these lacking any compound related trends and therefore were not considered to be of toxicological significance. At 6 months, the parameters affected in this way were haemoglobin concentration (♀, $p < 0,05$), haematocrit values (♀, $p < 0,05$), and leucocyte counts (♀, $p < 0,05$). Ar 12 months, mean cell volume (♀;10, 100 and 1000 ppm; $p < 0,05$) and mean cell haemoglobin (♀; 10, 100 and 1000 ppm; $p < 0,01$). At 18 months, leucocyte counts (♂, $p < 0,05$), and mean cell volume (♀, $p < 0,05$) and at two years, prothrombin time (♀, $p < 0,05$). In addition, AP activity was reduced in males exposed to 10, 100 and 1000 ppm compared to control. No correlation was noted between exposure level and the magnitude of the change in alkaline phosphatase values over a 100-fold range of exposure levels. The findings are not associated with any other deviation in the clinical chemical parameters, nor with evidence of any compound–related pathological changes. On the basis of available evidence, these findings are not considered to be of toxicological significance. Most changes seen in absolute and relative organ weight did not show consistent patterns and were not correlated with histopathological or clinical chemistry changes (Table 29). However, increased liver weight was associated with enzyme activity induction and increased kidney weight was associated with the increase in blood urea (Table 29). Histopathology: at 1 year and later, sciatic nerves showed very small numbers of nerve fibers exhibiting the changes of Wallerian degeneration (Table 37). Lesions consisted of swelling and fragmentation of axons and myelin. There was no difference in the intensity of the lesions in the proximal sciatic nerve and the distal. The appearances in the sciatic nerves were observed with the same incidence at all doses including the concurrent control animals. Statistical analysis (X^2) revealed no evidence of increased risk of tumor development over the 2 years period. The general health and behaviour of control and treated rats were similar throughout the 2 years study. Clinical signs were not compound related: Three male animals in the top dose 24 months group were removed from the trial in the first 5 weeks because of sores around the neck and in one case, diarrhea. Apart from this, the general health and behavior of control and treated groups were similar throughout the study. A variety of clinica signs were seen at all dose levels during the experiment and often resulted in animals being removed from the study and sent for necropsy for humanitarian reasons. The most common reasons for removal were the presence of sore hocks, gross external masses, mammary tumours or general poor conditions which was often associated with a loss of appetite. Observations are given in an descriptive way, no raw data or more detailed information is provided (timeframe, onset of clinical signs, distribution over the dose groups, …). The NO(A)EL in this study was 5 mg/Kg bw/d.

Table 37: Summary findings for 2 years 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%[n]	↘ 10%[n]
T0	135 (N=48)	122 (N=48)	134 (N=24)	121 (N=23)	137 (N=24)	122 (N=24)	133 (N=24)	124 (N= 24)	106** (N=21)	103** (N=24)
6 months	155 (N=48)	128 (N=48)	150 (N=24)	125 (N=23)	154 (N=24)	128 (N=24)	152 (N=24)	129 (N= 24)	145* (N=21)	120* (N=24)
12 months	140 (N=48)	118 (N=48)	139 (N=24)	111 (N=23)	139 (N=24)	113 (N=24)	145 (N=23)	114 (N= 23)	130* (N=21)	112 (N=24)
18 months	148 (N=46)	140 (N=41)	137 (N=21)	135 (N=21)	143 (N=23)	131 (N=23)	145 (N=23)	137 (N= 17)	146 (N=19)	136 (N=19)
24 months	112 (N=33)	95 (N=20)	97 (N=11)	78 (N=8)	102 (N=13)	83 (N=9)	108 (N=17)	96 (N= 10)	105 (N=17)	186 (N=12)
Mean Body weight									↘ 7% Week1 03	↘ 7% week 1-75
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
T0	129 (N=48)	131 (N=48)	126 (N=24)	129 (N=23)	129 (N=24)	131 (N=24)	126 (N=24)	130 (N= 24)	128 (N=21)	129 (N=24)
6 months	534 (N=48)	324 (N=48)	524 (N=24)	311 (N=23)	532 (N=24)	319 (N=24)	515 (N=24)	320 (N= 24)	490** (N=21)	296** (N=24)
12 months	574 (N=48)	387 (N=48)	581 (N=24)	379 (N=23)	578 (N=24)	387 (N=24)	566 (N=23)	392 (N=24)	530** (N=20)	358** (N=24)
18 months	569 (N=48)	403 (N=45)	582 (N=23)	402 (N=23)	582 (N=23)	393 (N=24)	565 (N=23)	419 (N=23)	534** (N=20)	375** (N=24)
24 months	552 (N=33)	412 (N=20)	560 (N=11)	384 (N=8)	560 (N=14)	413 (N=9)	524 (=17)	419 (N=12)	515* (N=17)	398 (N=13)
Abs;Rel. organ weight (g)										
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Testes									abs↘ 6 mth	

6 months	3.40; **ns** (N=12)	/	3.44;**ns** (N=6)	/	3.64; **ns** (N=6)	/	3.54; **ns** (N=6)	/	3.07**; **ns** (N=6)	/
12 months	3.50; **ns** (N=12)	/	3.68; **ns** (N=6)	/	3.63;**ns** (N=6)	/	3.33;**ns** (N=6)	/	3.39 ;**ns** (N=6)	/
18 months	3.42; **ns** (N=24)	/	3.79;**ns** (N=11)	/	3.70;**ns** (N=9)	/	3.80;**ns** (N=12)	/	3.69 ;**ns** (N=6)	/
24 months+	3.20;**ns** (N=32)	/	3.01;**ns** (N=11)	/	3.32;**ns** (N=13)	/	3.27;**ns** (N=17)	/	3.20 ;**ns** (N=17)	/
liver									abs+**re**l ↗ 18 mth	
6 months	16.24; **16.11** (N= 12)	9.93; **9.62** (N=12)	14.83; **15.28** (N=6)	9.72; **9.76** (N=6)	16.93; **15.23** (N=6)	9.80; **9.48** (N=6)	15.72; **15.94** (N=6)	9.81; **9.78** (N=6)	15.28; **16.58** (N=6)	9.32; **10.25** (N=6)
12 months	16.13; **16.40** (N= 12)	12.37; **12.09** (N=10)	18.33; **17.09** (N=6)	11.65; **11.77** (N=6)	17.70; **16.98** (N=6)	11.75; **11.47** (N=5)	16.79; **17.12** (N=6)	11.95; **12.07** (N=6)	16.70; **17.39** (N=6)	11.69; **12.28** (N=6)
18 months	16.58; **16.56** (N= 24)	13.71; **13.51** (N=17)	17.39; **17.16** (N=11)	14.45; **14.65** (N=9)	17.58; **17.13** (N=9)	13.48; **13.40** (N=8)	17.07; **17.91** (N=12)	14.05; **13.58** (N=10)	18.68** ; **19.56**** (N=12)	12.71; **13.21** (N=12)
24 months	16.63 (N=32)	12.79 (N=20)	15.59 (N=11)	12.28 (N=8)	16.75 (N=13)	12.59 (N=9)	16.13 (N=17)	11.89 (N=10)	16.45 (N=17)	13.33 (N=12)
heart									rel↗ 6 mth, abs↘ 12 mth	
6 months	1.27; **1.27** (N= 12)	0.95; **ns** (N=12)	1.25; **127** (N=6)	0.93; **ns** N=6)	1.39; **1.31** (N=6)	0.91; **ns** (N=6)	1.29; **1.29** (N=6)	1.13; **ns** (N=6)	1.29 **1.35*** (N=6)	0.87; **ns** (N=6)
12 months	1.37; **ns** (N= 12)	1.09; **1.06** (N=10)	1.41; **ns** (N=6)	1.07; **1.05** (N=6)	1.46; **ns** (N=6)	1.03; **1.00** (N=5)	138; **ns** (N=6)	1.05; **1.09** (N=6)	1.37; **ns** (N=6)	0.99*; **1.06** (N=6)
18 months	1.53; **1.53** (N= 24)	1.27; **ns** (N=17)	1.45; **1.44** (N=11)	1.25; **ns** (N=9)	1.57; **1.55** (N=9)	1.27; **ns** (N=8)	1.50; **1.49** (N=12)	1.28; **ns** (N=10)	1.57; **1.60** (N=12)	1.25; **ns** (N=12)
24 months	1.56 (N=32)	1.32 (N=20)	1.52 (N=11)	1.24 (N=8)	1.62 (N=13)	1.33 (N=9)	1.62 (N=17)	1.19 (N=10)	1.55 (N=17)	1.27 (N=12)
kidney							abs↗ 18 mth		rel↗ 12mth, abs↗ 18 mth	rel↗ 6 mth
6 months	3.18; **3.15** (N= 12)	1.91; **1.88** (N=12)	3.13; **3.15** (N=6)	1.96; **1.96** (N=6)	3.31; **3.20** (N=6)	1.95; **1.91** (N=6)	3.07; **3.10** (N=6)	1.98; **1.98** (N=6)	3.08; **3.18** (N=6)	1.96; **2.06*** (N=6)

12 months	3.08; **3.13** (N= 12)	2.30; 2.24 (N=10)	3.69; **3.48** (N=6)	2.24; **2.19** (N=6)	3.36; **3.24** (N=6)	2.27; **2.21** (N=5)	3.25; **3.31** (N=6)	2.30; **2.37** (N=6)	3.20; **3.39*** (N=6)	2.22; **2.37** (N=6)
18 months	3.74; **ns** (N= 24)	3.16; **ns** (N=17)	3.68; **ns** (N=11)	3.12; **ns** (N=9)	3.92; **ns** (N=9)	2.52; **ns** (N=8)	4.28*; **ns** (N=12)	2.71; **ns** (N=10)	4.01*; **ns** (N=12)	2.65; **ns** (N=12)
24 months	3.80 (N=32)	3.01 (N=20)	3.74 (N=11)	2.08 (N=8)	4.05 (N=13)	2.93 (N=9)	4.11 (N=17)	2.66 (N=10)	4.33 (N=17)	2.80 (N=12)
Clinical chemistry:										
Urea (mmol/l)									↗ 58% 2 y	
6 months	7.12	7.93	7.35	7.37	6.80	7.35	7.67	8.05	7.40	8.40
12 months	7.0	9.0	6.7	8.8	6.8	8.9	7.5	8.2	7.5	9.7*
18 months	7.9	9.3	8.5	7.3	7.4	7.4	9.2	8.0	8.6	8.4
24 months	7.8	8.7	7.4	6.7	8.1	6.1	11.3	6.9	12.3	7.0
AP (IU)					↘ 16% 2 y		↘ 18% 2 y		↘ 33% 2 y	
6 months	72	49	60	44	60	42	69	53	55	43
12 months	86	60	78	53	75	60	76	58	83	55
18 months	76	47	79	36	70	37	63	38	73	38
24 months	72	37	63	32	53*	35	59*	36	56*	40
Na^+ (mmol/l)									↗ 1%	
6 months	145	144	145	144	147	144	146	144	146	144
12 months	146	144	145	146	146	145	146	145	146	146
18 months	147	145	147	143	147	144	147	143	147	144
24 months	145	144	145	144	145	145	145	144	146*	143
Histopathology: sciatic nerve degeneration : number affected/number survivors										
at 12 mth	2/12	1/12	n.r.a	n.r.a	n.r.a	n.r.a	0/6	2/6	1/6	2/6
at 18 mth	9/24	2/17	n.r.a	n.r.a	n.r.a	n.r.a	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	n.r.a	n.r.a	n.r.a	n.r.a	14/35	9/24	18/35	7/30
%	42	26.5	n.r.a	n.r.a	n.r.a	n.r.a	40	37.5	51.4	23

*Stat. significant ↗ or↘ : covariance analysis; variance analysis; Williams t test or Dunnett's test ($P < 0.05$)
** Stat. significant ↗ or↘ : covariance analysis; variance analysis; Williams t test or Dunnett's test ($P < 0.01$)
[n] not statistically significant
ns: No significant covariance relationship; original value not reported in the study
(N=) number of observations
[+]Adjusted for initial body weight
ABS;**REL**= Mean weight organ; **mean weight organ adjusted for terminal body weight** (g)
After 2 years, organ weight adjusted for terminal body weight were all non-significant.

Non-rodent

From the results of the chronic toxicity study in the rat [redacted], (1978) and comparing this against the 90-day subchronic toxicity study ([redacted]1976), there is no apparent change in the actual No Observed Adverse Effect Levels (see section above). 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 ([redacted]. 2006) 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 [redacted], (1977) also show 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 minimize 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.

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

Selected toxicological findings and the criteria for classification for Specific target organ toxicity – repeated exposure are summarised in Table 38.

Neurotoxic effects:
All available date is shown in chapter 4.7 and chapter 4.8. Unfortunately, in these old regulatory studies no raw data of the clinical signs were made available, nor was this data reported in tables. The only clinical observations data available is given in a descriptive way. There is no more information available concerning the time of appearance and disappearance of the effects, the duration of the effects, after how many repeated doses, time elapsed between the exposure and the clinical effects, the intensity and severity of the reported neurotoxic effects.

Furthermore, except for 1 dermal repeated dose study, only repeated dose studies performed by oral administration are available and can be taken in consideration.

Table 358: Selected toxicological results (at dose levels below the guidance values) in comparison with criteria of Specific target organ toxicity – repeated exposure

Toxicological result	CLP criteria
Key studies:	
5-week oral study in dogs ([redacted], 1976): **Rel= 3** Dose: 0, 15, 150, 1500 ppm, equivalent to 0, 0.375, 3.75, 37.5 mg/kg bw/d Results: 37.5 mg/kg bw/d: **Neurotoxicity** apprehension, diarrhoea, vomiting, licking and chewing of the paws, whole body tremors and stiff exaggerated hind leg gait, ataxia. 2 animals convulsed during week 1 and 5. ↓ bw gain ⓿ rel. thyroid weight	Category 1 (H372): Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations. Equivalent guidance values for different study durations: Oral, rat: 28-day: ≤ 30 mg/kg bw/d 35-day: ≤ 25 mg/kg bw/d 90-day: ≤ 10 mg/kg bw/d

⓿ WBC and KCCT at week 5 in ♂; ⓿ blood urea levels, ↓ blood glucose levels at week 5 in ♀ NO(A)EL: 3.75 mg/kg bw/d LO(A)EL: 37.5 mg/kg bw/d NO(A)EL would lead to cat1, however, at lower conc no substance-related changes -> cat2	1-yr: ≤ 2.5 mg/kg bw/d 2-yr: ≤ 1.25 mg/kg bw/d Dermal, rat or rabbit: 28-day: ≤ 60 mg/kg bw/d 90-day: ≤ 20 mg/kg bw/d
90-d oral study in dogs ([redacted], 1977): **Rel=3** Dose: 0,5,50,500,1500 ppm, equivalent to 0, 0.125, 1.25, 12.5, 37.5 mg/kg bw/d <u>Results: 37.5 mg/kg bw/d:</u> **Neurotoxicity** Diarrhea, licking and chewing of the paws, whole body tremors, stiff exaggerated hind leg gait, ataxia, incoordination and hyperaesthesia. ↓food intake and ↓bw 4 dogs sacrificed for humane reasons ♀↓RBC), ↓KCCT (kaolin-cephalin clotting time) Focal bronchopneumonia NO(A)EL: 12.5 mg/kg bw/d LO(A)EL: 37.5 mg/kg bw/d ->cat2	Category 2 (H373): Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure. Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. In exceptional cases human evidence can also be used to place a substance in Category 2. Equivalent guidance values for different study durations: Oral, rat: 28-day: 30 < C ≤ 300 mg/kg bw/d 35-day: 25 < C ≤ 250 mg/kg bw/d 90-day: 10 < C ≤ 100 mg/kg bw/d 1-yr: 2.5 < C ≤ 25 mg/kg bw/d 2-yr: 1.25 < C ≤ 12.5 mg/kg bw/d Dermal, rat or rabbit: 28-day: 60< C ≤ 600 mg/kg bw/d 90-day: 20 < C ≤ 200 mg/kg bw/d
Supporting information:	
90-d oral study in rats ([redacted], 1976): **Rel=2** Dose: 0,25,100,400,1600 ppm, equivalent to 0, 1.25, 5, 20,80 mg/kg bw/d <u>Results: 80 mg/kg bw/d:</u> **Neurotoxicity** Hypersensitivity to sound and touch, abnormal gait (ataxia and splayed hind limbs) during the first 5 weeks (9/12 males, 7/12 females). Mortality: 1 died, 3 killed. 2 of these rats showed axon breaks and vacuolation of myelin in the sciatic nerve. ↓BW, ↓food intake, ↓Hb, ↑urea, ↑kidney weight ♂ ↓KCCT, ↑K^+ ♀↓RBC, ↑AP, ↑liver weight, ↑spleen weight NO(A)EL: 20 mg/kg bw/d LO(A)EL: 80 mg/kg bw/d ->cat2	

90-d oral study in rats (*Varshneya et al,* 1992): **Rel= 3** Dose: 0, 5, 10, 20, 40 mg/kg bw/d Results: **Immunotoxicity** Dose-dependent ↓ in delayed type hypersensitivity reaction on d61 post treatment 20 mg/kg bw/d: ↑adrenal weight 40 mg/kg bw/d: ↓spleen weight, ↑adrenal weight, leucopenia on d90 NO(A)EL: 10 mg/kg bw/d LO(A)EL: 20 mg/kg bw/d ->cat2 Clinical signs: No clinical signs/neurotoxicity reported	
24 months oral study in rats ([redacted], 1978): **Rel= 2** Dose: 0, 1, 10, 100, 1000 ppm, equivalent to 0, 0.05, 0.5, 5, 50 mg/kg bw/d Results: <u>50 mg/kg bw/d:</u> ↓food consumption & bw ↑platelets ↑liver PNOD, ♂↑urea, ♂↓AP Organ weight ↑: liver abs, rel; heart, rel; kidney, rel, abs Organ weight↓: testes, rel; heart, abs NO(A)EL: 5 mg/kg bw/d LO(A)EL: 50 mg/kg bw/d Not enough for direct classification but supportive for the weight of evidence approach due to significant effects on organs and biochemical parameters. Clinical signs: Three male animals in the top dose 24 months group were removed from the trial in the first 5 weeks because of sores around the neck and in one case, diarrhea. Apart from this, the general health and behavior of control and treated groups were similar throughout the study. A variety of clinica signs were seen at all dose levels during the experiment and often resulted in animals being removed from the study and sent for	

necropsy for humanitarian reasons. The most common reasons for removal were the presence of sore hocks, gross external masses, mammary tumours or general poor conditions which was often associated with a loss of appetite. Observations are given in an descriptive way, no raw data or more detailed information is provided (timeframe, onset of clinical signs, distribution over the dose groups, …)	
12 weeks oral study in rabbits (*Yousef et al,* 2003): **Rel= 3** Dose: 0, 24 mg/kg bw every other day Results: ↓bw gain ↑rel. liver, spleen, kidney weight ↑plasma glucose, urea, creatinine, total bilirubine ↓plasma total protein, albumin ↑plasma total lipid, cholesterol, TG, LDL, VLDL ↓HDL ↓Hb, RBC, PCV, ↑total leucocyte count NO(A)EL: <24 mg/kg bw/every other day LO(A)EL: 24 mg/kg bw/every other day ->cat2 Clinical signs: No clinical signs/neurotoxicity reported	
5-week oral study in rats ([redacted],1976): **Rel= 3** Dose: 25,100,250,750,1500 ppm, equivalent to 1.25,5,12.5,37.5,75 mg/kg bw/d <u>Results: 75 mg/kg bw/d</u>: **Neurotoxicity** Piloerection, nervousness, uncoordinated movements from week 2 onwards. bw gain, food intake, terminal bw reduced organ weight: ⓿ abs and rel liver weight in ♀ ⓿ haemoglobin and blood urea conc. in ♂; ⓿ plasma alkaline phosphatase in ♀ NO(A)EL: 37.5 mg/kg bw/d LO(A)EL: 75 mg/kg bw/d ->cat2	
3-week oral study in rats (*El Toukhy et Girgis* 1993): **Rel=** Liver toxicity at 31.5 mg/kg bw/d	

Inhibition of liver ATPase	
5-d oral study in rats (*Girray et al,* 2001): **Rel= 3** Liver toxicity and neurotoxicity (abnormal gait and nervousness) at 75 mg/kg bw/d Enhanced lipid peroxidation in hepatic and cerebral tissues	
3-week (5d/week) dermal study in rabbits ([redacted], 1981): **Rel= 3** Dose: 0,2,20,200 mg/kg bw/d Results: 200 mg/kg bw/d: ↓food intake, bw gain, weight of gonads Focal liver necrosis NO(A)EL: 20 mg/kg bw/d LO(A)EL: 200 mg/kg bw/d Extrapolation, however, at lower concentration no substance-related changes other than local effects on skin -> cat2	
7-d intraperitoneal study in rats (*Aldana et al,* 1998): **Rel= 3** Liver toxicity and neurotoxicity at 300 mg/kg bw/d Neurotoxicity: animal behaviour = scratching, salivation, somnolence, ataxia, convulsion and hind limb paralysis noted at every time point, on a daily basis during 7 days Liver: histology	

The **short/medium-term oral toxicity** of cypermethrin was studied in rats and dogs.

Dogs were the more sensitive species to cypermethrin toxicity. In the dog, a 90-day oral toxicity study of cypermethrin at doses of 0, 0.125, 1.25, 12.5, and 37.5 mg/kg bw/day was performed ([redacted], 1977). Clinical signs of neurotoxicity were observed at 37.5 mg/Kg bw/d : diarrhea, licking and chewing of the paws, whole body tremors, a stiff exagegerated hind leg gait, ataxia, incoordination and hypereasthesia. No overt toxicity (clinical signs, haematological and clinical chemistry parameters, pathological effects) was observed in the dose groups up to 12.5 mg/Kg bw/d (NO(A)EL = 12.5 mg/Kg bw/d) The 5-week short term oral toxicity study in dogs ([redacted],1976) resulted in similar symptoms. Administered doses were 0, 0.375, 3.75, and 37.5 mg/kg bw/day. Clinical signs were observed at a dose of 37.5 mg/Kg bw/d, characterized by apprehension, diarrhoea, vomiting, licking and chewing of the paws, whole body tremors and stiff exaggerated hind leg gait, and ataxia.. The NO(A)EL oral, dog, 5 weeks is 3.75 mg/Kg bw/d. The neurotoxicity effects observed at 37.5 mg/kg bw/day in the 90-day and 5-week study are between the classification cut offs, setted for the rat, for **STOT RE2** of 10<C ≤100 mg/kg bw/day and 25<C≤250

mg/kg bw/day, respectively and can thus be used for direct classification or as supportive date for the weight of evidence approach**.**

Rats were fed a diet containing cypermethrin at concentrations of 0, 1.25, 5, 20 and 80 mg/Kg bw/d over a period of 90 days ([redacted], 1976). In this study, clinical signs of neurotoxicity observed at 80 mg/Kg bw/day were hypersensitivity and abnormal gait during the first 5 weeks of the experiment. In rats, neurotoxicity was confirmed by histopathology as peripheral nerve damage: two rats showed axon breaks and vacuolation of myelin in the sciatic nerve. The health and behaviour of animals in the dose group up to 20 mg/Kg bw/d were unaffected by ingestion of cypermethrin. (NO(A)EL = 20 mg/Kg bw/d). In the 5-week oral toxicity test ([redacted], 1976), with administered doses of 1.25, 5, 12.5, 37.5 and 75 mg/kg bw/day, a dose of 75 mg/Kg bw/d resulted in clinical signs of toxicity including piloerection, nervousness and uncoordinated movements from week 2 onwards. NO(A)EL oral, rat, 5 weeks = 37.5 mg/Kg bw/d. In these rat studies also, effective dose levels are between the classification cut offs for **STOT RE2.**

Some short-term open literature studies confirm the neurotoxic effect of cypermethrin. In the study by *Aldana et al.* (1998), intraperitoneal administration to rats of 300 mg cypermethrin/kg bw/day for 7 days resulted in scratching, salivation, somnolence, ataxia, convulsion and hind limb paralysis. In a 5-day oral study in rats, *Giray et al.* (2001) noted no clinical signs at 75 mg/kg bw/day; however, cerebral tissues did show enhanced oxidative stress.

In the available long-term study, neurotoxicity was less apparent. In a 24-month oral study ([redacted], 1978) cypermethrin was administered to rats in the feed at doses of 0, 0.05, 0.5, 5.0, and 50 mg/Kg bw/d. At these concentrations, no test substance related mortalities or signs of clinical toxicity were observed. At 1 year and later, sciatic nerve degeneration was evident, however these symptoms were observed with the same incidence at all doses including controls.

Other effects were noted in regulatory studies and literature, yet none of these were sufficiently substantiated to conclude on specific routes for classification as STOT RE. However, some of these studies indicate the liver as a target organ for cypermethrin toxicity.

In the 5-week oral toxicity study in dogs ([redacted], 1976), at 37.5 mg/kg bw/day body weight gain was reduced due to the observed loss of apetite. In females, blood urea concentrations were increased and blood glucose levels decreased at week 5. In males, white blood cell count (WBC) and kaolin-cephalin clotting time (KCCT) values were increased at week 5. Relative thyroid weight was increased in both males and females. In rats, the 5-week oral toxicity study ([redacted], 1976) showed reduced food consumption, body weight gain and terminal body weight in both males and females at 75 mg/kg bw/day. In females, an increase in relative liver weight was observed and an increase in plasma alkaline phosphatase activity. In males, hemoglobin and blood urea concentrations were increased.

A 21-day dermal toxicity study in rabbits ([redacted], 1981) illustrates the hepatotoxic effect of cypermetrin. This study was performed on abraded skin, under occluded patch; administered doses were 0, 2, 20 and 200 mg/kg bw/day. At the top dose, the irritation of the skin was associated with focal liver necrosis as a systemic effect. The liver necrosis did not show a clear zonal distribution within the lobules, although many involved the periportal zone. (NO(A)EL = 20 mg/Kg bw/d).

The open literature also demonstrates hepatotoxicity. At 300 mg/Kg bw/d (i.p., 7 days) in rats, hepatotoxicity was evident from histological findings in the study by *Aldana et al.* (1998). *El-Toukhy and Girgis*,(1993) showed that rat liver ATPase was inhibited after a 3 week oral treatment with 31.5 mg/Kg bw/d. Oral administration of 75 mg/kg bw/day resulted in oxidative stress in rat hepatic tissues

induced by cypermethrin exposure: levels of thiobarbituric acid reactive substances (TBARS) and conjugated dienes were elevated (*Giray et al*., 2001).

In the subchronic oral rat study ([redacted], 1976), body weight gain was reduced at the top dose (80 mg/kg bw/day) throughout the experiment, and in females, liver weight was increased. An increase in kidney (m/f) and spleen (f) weight was also observed. Plasma urea was increased and haemoglobin was decreased. In addition, in males KCCT was decreased and Kalium was increased. In females RBC count was decreased, alkaline phosphatase was increased. In dogs, reduced food intake and body weight gain were noted at 37.5 mg/kg bw/day in the 90-day oral study as well ([redacted], 1977). RBC count and KCCT were decreased in the female dogs. Non-specific pathological changes, mainly in the lung, were also found.

Subchronic open literature studies reported moderate toxic effects on hemato-biochemical functions including hematological parameters and profiles of lipid, lipoproteins, protein, urea, creatinine, glucose and total bilirubin in rabbits when exposed to 24 mg cypermethrin/kg bw every other day for 12 weeks (*Yousef et al.*, 2003a). In rats, a 90-day oral exposure to cypermethrin led to immunotoxicity: it induced a dose-dependent decrease in delayed type hypersensitivity (DTH) reaction. The top dose of 40 mg/Kg bw/d resulted in a leucopenic response (*Varshneya et al.*, 1992).

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

Regarding the effects observed in the short- and medium-term oral studies (there is only 1 dermal 3-week study and 1 intraperitoneal 7-d study apart from the oral studies), a classification as STOT RE2 for neurotoxicity is warranted. Short- and medium-term repeated dose toxicity studies in dogs are to be regarded as key studies. Supporting studies in rats and open literature studies are also available.

The **short/medium-term oral toxicity** of cypermethrin was studied in rats and dogs. The central nervous system was detected as the target tissue. Neurotoxicity was characterized by clinical signs including piloerection, nervousness and uncoordinated movements, ataxia, splayed gait and hyperesthesia. In rats, neurotoxicity was confirmed by histopathology as peripheral nerve damage. The neurotoxicity-related clinical signs were observed at effective dose levels between the classification cut offs for **STOT RE2.** Open literature confirmed neurotoxicity through clinical signs in rats at high cypermethrin doses. The oxidative stress induced by cypermethrin in cerebral tissues was evidenced by enhanced lipid peroxidation.

Classification/Labelling of the active substance ‘cypermethrin’ for repeated-dose toxicity according to the criteria in CLP-regulation (EC) No 1272/2008:

STOT RE 2, H373 (nervous system). May cause damage to the nervous system through prolonged or repeated exposure.

It cannot be excluded that STOT-RE 2 also applies for other routes of exposure (dermal, inhalation). There is no scientific data available to conclusively show that no classification for STOT-RE is warranted for other routes than the oral route of exposure.

Other effects noted in regulatory studies and literature indicate the liver as a target organ for cypermethrin toxicity. However, none of these studies were sufficiently substantiated; they do not fulfil the criteria for STOT RE classification.

4.9 Germ cell mutagenicity (Mutagenicity)

Not evaluated in this dossier.

4.10 Carcinogenicity

Not evaluated in this dossier.

4.11 Toxicity for reproduction

Not evaluated in this dossier.

4.12 Other effects

None

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

Table 39: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Hydrolysis; EEC method C.7 GLP = Yes Rel = 1	pH4; 50°C; DT_{50} > 1year pH7; 50°C; DT_{50} = 4.73 days pH9; 50°C; DT_{50} = 1.9 hours pH7; 25°C; DT_{50} > 29 days	Main hydrolysis degradants: DCVC acid and 3-phenoxybenzaldehyde formed in equimolar amounts. The latter will probably oxidise readily to 3-phenoxybenzoic acid.	*Schneider*, 1997, Krebs Analytic GmbH, report no. PR97/003 CYP/C52
Photolysis in water; EC directive 94/37/EC Section 2.9.2 GLP = Yes Rel = 1	Total recovery of test substance 68% of applied substance after 90 hours. Reaction quantum yield 0.0308	Initial concentration 0.004 µg/mL	*Greenwood and Mausley*, 2003, Covance report no 0040/034
Photolysis in water; EC directive 94/37/EC Section 2.9.2 GLP=Yes Rel=1	(^{14}C Phenoxy) cypermethrin - DT_{50}= 14.7 - Direct photolysis rate constant (per day) = 0.0469 (^{14}C cyclopropane) cypermethrin - DT_{50} = 12.4 - Direct photolysis rate constant (per day) = 0.0557	Half-life (d), dark control 22.1 d 16.5 d	*Swales*, 2003a, Covance, report no. 40/35-D2149 CYP/M70.Greenwood, 2003, Covance report no 0040/034
Photolysis in soil; EC directive 94/36/EC GLP = Yes Rel = 1	DT_{50}= 3 days (phenoxy label) 2.5 days (cyclopropane label)	The half-life is based on all the cypermethrin the soil being exposed to light. In reality only material on the surface will undergo photodegradation. The rest will be degraded as per dark control.	*Swales*, 2003b,Covance report number 40/44,
Ready Biodegrdability (modified Sturm test) OECD301B GLP = Yes Rel = 1	0.6-1.4% degradation after 33 days		Klein, Fraunhofer-institut.1990. Report no FE1-001/3-11
Inherent biodegradability: Used Methodology of OECD 301B but using medium composition, test substance and inoculum concentrations from OECD 302C. GLP = Yes Rel = 1	0% degradation within 28 days	The test substance is slightly toxic to the activated sludge microbes.	*Burwood*, 2005,Covance Report no 1669/017-D2149
Ultimate degradation: anaerobic BS method 6068 and ISO method 11734 (1995) GLP = Yes Rel = 1	17% degradation within 60 days	Indicative value due to lack of radiolabelled monitoring of degradation.	*Barnes*, 2005, Huntingdon Life Sciences, HZL 010/053287
Aerobic and anaerobic water-sediment simulation study OECD308 Rel = 2	After 100 days, the DT-90 in both systems had been exceeded and all known metabolites that individually comprised > 5% of applied radioactivity were in decline. An unidentified metabolite was still increasing after 100 days. Cypermethrin degraded very rapidly in both water-sediment systems. DT-50 values were between 3.5 (SFO Kinetic) and 9.8 days in each total sediment system. Dissipation from the water phase was more rapid than from the system as a whole (DT-50 values were 0.5 days for both systems). Dissipation from the sediment phase was characterized by DT_{50} comprised between 10.9-14.3 days.		*Brice* 2006a Covance report 1669/014,

	The metabolites of cypermethrin degraded extensively by mineralization to carbon dioxide. Mean levels of carbon dioxide evolved were over the range 25-69% of applied radioactivity at 100 days. Dissolved carbon dioxide was also present in the water and sediment phases. 3 major metabolites formed, of which 2 with a DT_{50} > 40 days.		
Aerobic degradation in soil OECD307 GLP=yes Rel=1	DT_{50} of the whole system between 6.4 and 24.2 d at 20°C (corrected to 12°C : DT_{50} between 12.13 and 45.9 days, geom.mean =17.2 days) 3 metabolites		*Brice A and Cooke C.*, 2006c, Covance study report 1669/013- D2149.
Anaerobic degradation in soil OECD 307 (April 2002) GLP = Yes Rel =1	The DT_{50} total cypermethrin 46 days at 20°C (corrected to 12°C; DT_{50} =87.2 days) 3 metabolites		*Brice A, Cooke, C.*, (2006d), Covance study report 1669/013- D2149.

5.1.1 Stability

Hydrolytic degradation

Table 40: Hydrolytic degradation study of cypermethrin cis/trans +/- 40/ 60

Guideline/ Test method	pH	Temperature (ºC)	Initial concentration (µg/L)	Reaction rate constant, Kh	Half-life, DT_{50} (days)	References
EEC method C.7 GLP = Yes Rel= 1	4	50	100 µg/L	Not determined	> 1 year.	*Schneider*, 1997, Krebs Analytic GmbH, report no. PR97/003 CYP/C52
	7	50	100µg/L	Not determined	4.73 days	
	9	50	100µg/L	0.017	1.9 hours	
	7	25	100µg/L	0.007	> 29 days.	
Main hydrolysis degradants: DCVC acid and 3-phenoxybenzaldehyde formed in equimolar amounts. The latter will probably oxidise readily to 3-phenoxybenzoic acid.						

Schneider, (1997) has investigated the stability of cypermethrin cis:trans/40:60 with respect to hydrolysis behaviour in water at pH 4, pH 7 and pH 9 according to EEC method C7. Test samples were prepared using an initial concentration of 100 µg/L cypermethrin (cis:trans/40:60). Test vials were maintained at 50 ºC with the exception of one of the two pH 7 vials, which was kept at room temperature (23-26°C). The extracts were anaylsed by HPLC to determine the concentration of parent compound. Metabolites were identified by comparison with known reference substances and the mass balance calculated. Where hydrolysis occurred, the half-life was calculated using the IVA computer model.

The study shows that cypermethrin cis/trans +/- 40/ 60 hydrolysis is pH dependant. Cypermethrin cis/trans +/- 40/ 60 can be regarded as relatively stable in acidic condition with no apparent degradation up to 29 days and unstable in alkaline media with a determined half-life of 1.9h at 50°C. At neutral pH (pH= 7) cypermethrin cis/trans +/- 40/ 60 has a half-life of 4.73 day at 50°C. However, according to the photolysis study (see below) dark controls at 20±3°C show hydrolysis reaction

between 12 to 18 % after 4 days. According to the equation 25 from the TGD on Risk Assessment, part II ($DT_{50}(X\,°C) = DT_{50}(t) \cdot e^{(0.08 \cdot (T-X))}$), the respective half-life corrected for 12°C becomes 98.8 d for pH 7 and 1.65 day at pH 9. According to these last values, the respective pseudo first-order rate constant can be calculated to be KpH 7 = 0.007 $d^{-1}$and KpH 9= 0.42d^{-1}.

Conclusion:

Conclusion for hydrolysis: Cypermethrin cis:trans/40:60 will hydrolyse more rapidly in alkaline conditions than acidic conditions. Under neutral pH (7) at temperature of 25° it is stable for at least 29 days (test period).

Photolytic degradation in water

Photolysis in water was addressed in two independent studies on cypermethrin cis/trans +/- 40/ 60 by *Greenwood, J.and Maudsley, L.* (2003) and by *Swales, S.* (2003).

Table 41: Photolytic degradation of cypermethrincis/trans +/- 40/ 60 in water (*Greenwood*, 2003)

Guideline/ Test method	Initial concentration	Total recovery of test substance (% of applied)	Reaction Quantum yield	Reference
EC directive 94/37/EC Section 2.9.2 GLP = Yes Rel = 1	0.004 µg/mL	68% after 90 hours	0.0308	*Greenwood J. And Mausley L.*, 2003, Covance report no 0040/034

In the first study, by *Greenwood and Maudsley,* (2003), the quantum yield for direct photolysis of [^{14}C-cyclopropane] cypermethrin (cis:trans 40:60) in sterile pH4 aqueous buffer was determined after 90 hours continuous irradiation with artificial sunlight using a Xenon lamp. The photon flux as a function of wavelength of the light source was measured using a spectroradiometer. A binary chemical actinometer solution, of known quantum yield, was simultaneously irradiated with the test samples. The quantum yield was then calculated from the information generated.

Analysis of the buffer extracts units by Thin Layer Chromatography (TLC), illustrated that after 90 hours irradiation, the majority of radioactivity detected was cypermethrin, with a mean of 68% of applied radioactivity at 90 hours in the irradiated units and 95% in the dark control units. The levels of material found at the origin of the chromatogram corresponding to metabolites or degraded cypermethrin increased throughout the study, reaching 16% and 2% of applied radioactivity at 90 hours in the irradiated and dark controls units respectively, confirming that photodegradation of cypermethrin had taken place.

Table 42: Photolytic degradation of cis/trans +/- 40/ 60 in water (*Swales*, 2003)

Guideline/ Test method	Isomer	Initial conc.	Total recovev. of test substance (% of applied)	Photolysis rate constant (Kcp)	Direct photolysis rate constant (per day)	Half-life (t½E) (days)	Half-life (d), dark control	Reference
EC directive 94/37/EC	(^{14}C Phenoxy) cypermethrin	0.004µg/mL	96-105%.	0.078	0.0469	14.7	22.1	*Swales*, 2003, Covance,

Section 2.9.2 GLP = Yes Rel = 1	(^{14}C cyclopropane) cypermethrin	0.004µg/mL	96-107%	0.0976	0.0557	12.4	16.5	report no. 40/35-D2149 CYP/M70.Greenwood, 2003, Covance report no 0040/034

In the second study by *Swales* (2003) the photodegradation rate of [^{14}C] cypermethrin (cis:trans 40:60) was studied at 20°C in pH 4 buffer (sterile conditions, < 1% acetonitrile co-solvent) with continuous irradiation for up to 100 hrs (equivalent to ca. 7 days of summer sunlight).

Degradation process was first order in both the irradiated solution and controls. The half-life in the irradiated solution was 8.85 summer sunlight days for the (^{14}C phenoxy) cypermethrin and 7.10 summer sunlight days for the (^{14}C cyclopropane) cypermethrin. The corresponding dark control samples had half-lives of 22.1 and 16.5 days respectively. All figures are quoted as equivalent to Florida summer sunlight days. From the rate constants obtained for irradiated samples and dark controls, the net photolysis rate constant and corresponding half-lives were calculated to be 0.0469 d^{-1} and 14.7 days for ^{14}C phenoxy label and 0.0557 d^{-1} and 12.4 days for ^{14}C cyclopropane label. The main photolytic degradants were 3-Phenoxybenzoic acid (15%), DCVC acid (18%) and 3-phenoxybenzaldehyde (max levels were 3%) of applied radioactivity. A further 16 unidentified photolytic degradation products containing < 10% of applied radioactivity at any time point (maximum 5.6% at 7 day sunlight equivalent) were detected.

Photolytic degradation in soil

Table 363: Photolytic degradation of cypermethrin cis/trans +/- 40/ 60 in soil, (*Swales*, 2003)

Guideline / Test method	Initial concent-ration	Total recovery of test substance (% of applied)	Irradiation time	Photolysis rate constant (Kcp)	Half-life (t½E)	Remarks	Reference
EC directive 94/36/EC GLP = Yes Rel= 1	Equiv. to 25g/ha	Overall recovery of radioactivity was 95 to 106%	15 days continuous	0.231 per day (phenoxy label) 0.276 per day (cyclopropane label)	3 days (phenoxy label) 2.5 days (cyclopropane label)	The half-life is based on all the cypermethrin cis/trans +/- 40/ 60 applied to the soil exposed to light. In reality only material on the surface will undergo photodegradation, the rest will be degraded as per dark control.	*Swales*, 2003,Covance report number 40/44,

The photodegradation of (^{14}C phenoxy) cypermethrin and (^{14}C cyclopropane) cypermethrin, cis:trans ratio 40:60 was investigated by Swales (2003) on thin layers of a silty clay loam soil exposed to simulated sunlight, filtered to remove wavelengths below 290 nm. The soils layers were formed by

spreading slurry on the soil on metal trays and allowing the soil to dry at 35°C. [^{14}C phenoxy] cypermethrin (40/60) and [^{14}C cyclopropane] cypermethrin (40/60) were applied to the surface of the soil at the rate of 25 g/ha, based on the surface area of the soil dish.

The temperature of the test soil was maintained at 20 ± 3°C. Additional samples were kept in a temperature controlled dark incubation room. All test vessels were connected with traps to absorb volatile compounds. The extracts were concentrated and quantified by LSC. Bound residues and trapping solutions were quantified by LSC. All samples were analysed by HPLC and selected samples were analysed by TLC for confirmation.

In irradiated soil, the major degradation product is the carboxamide derivative of cypermethrin (19% AR after 7-9 days continuous irradiation) along with smaller amounts of 3-phenoxybenzoic acid (3% AR) and DCVC acid ((2,2-dichlorovinyl)- 2,2- dimethylcyclopropanecarboxylic acid) (6% AR). Bound residue reached 12.8-21.9 % AR at day 15, mineralisation reached 5.4-6.2 % AR at day 15.

In dark samples, the major degradation products are 3-phenoxybenzoic acid (24% AR) and DCVC (13 %AR); carboxamide is formed at a lower level (5% AR).

Bound residue reached 10.6-10.7% AR at day 15, mineralisation reached 0.2-2.5 % AR at day 15.

Using a two-phase decay curve the DT_{50} values were 29.6 days and 43.9 days (light samples) and the DT_{90} values were 201 and 230 days (light samples) for the (^{14}C phenoxy) cypermethrin and the (^{14}C cyclopropane) cypermethrin, respectively. A two-phase decay was used as there was an initial rapid degradation, followed by a slower phase, which may indicate that only a proportion of the applied cypermethrin was on the soil surface and could undergo photolysis. For this reason a half-life was calculated using the first order rate constant from the initial rapid portion of the two-phase degradation curve. This method of calculation resulted in half-life values of 3 days for the (^{14}C phenoxy) cypermethrin and 2.5 days for the (^{14}C cyclopropane) cypermethrin.

Conclusion: Light accelerates the degradation of cypermethrin (cis:trans/40:60) on a soil surface, in the air and in water. However data on distribution of radioactivity and DT_{50} for cis- and trans- isomers indicate that soil photolysis is a minor route of degradation of the active substance.

5.1.2 Biodegradation

Table 374: Summary of biodegradation studies on cypermethrin

Guideline/ Test method	Test type	Inoculum		Test substance concentration.	Degradation		Reference
		Type	Adaptation		Incubation period	Degree (%)	
OECD 301B GLP = Yes Rel = 1	Ready Biodegrdability (modified Sturm test)	Activated sludge, domestic	No	10 mg/L and 20 mg/L	35 days	0.6-1.4% at 33 days.	Klein, Fraunhofer-institut.1990. Report no FE1-001/3-11
Used Methodology of OECD 301B but using medium composition, test substance and inoculum concentrations	Inherent biodegradability.	Activated sludge	No	30 mg/L	28 days	0	*Burwood,* 2005,Covance Report no 1669/017-D2149

from OECD 302C. GLP = Yes Rel = 1							
BS method 6068 and ISO method 11734 (1995) GLP = Yes Rel = 1	Anaerobic (ultimate) biodegradability	Mineral salts medium (MSM) and pre-digested anaerobic sludge inoculum, domestic	No	20 mg as [C]/L	60 days	17%*	*Barnes*, 2005, Huntingdon Life Sciences, HZL 010/053287

* Indicative value, see discussion below

Ready degradability

Cypermethrin (not further specified) was tested for the ready biodegradability by Klein, in 1990 using the modified sturm test (OECD 301B). Initial cypermethrin ((RS)- á -cyano-3-phenoxybenzyl-(1R,1S)-cis, trans-3-(2.2-dichlorovinyl)-2.2-dimethyl-cyclopropan-carboxylat) concentrations of 10 and 20 mg a.s./L were used. During the test, the vessels were aerated with CO_2-free air. The extent of biodegradation was determined by titration of the total CO_2 evolved during the incubation for 33 days. Aniline was used as a reference substance to ensure validity of the test (degradation being > 60% over 28 days). An innoculum blank was also used to calculate CO_2 generated from the test substance.
The biodegradation of cypermethrin was 0.6 to 1.4% after 33 days incubation, whereas biodegradation of aniline (positive control) was 94.4 to 100.7% after 28 days incubation, indicating that the inoculum was effective.

In conclusion, cypermethrin (not further specified) is **not readily biodegradable** according to the test criteria.

Inherent biodegradability

In a second biodegradability tests, cypermethrin cis:trans/40:60 was tested by *Burwood C.,* (2005) to check the inherent biodegradability. The inherent biodegradability of Cypermethrin cis:trans/40:60 was assessed by measuring carbon dioxide evolution. The study adopted the methodology of the OECD Guideline 301B, CO_2 evolution test but used the medium composition and test substance and inoculum concentrations from OECD Guideline 302C.

The test material was suspended in a buffered mineral salts medium at a nominal concentration of 30 mg/L. The medium was inoculated with microorganisms derived from a sample of a non-adapted activated sludge. Test vessels were incubated for 28 days and the medium continually sparged with a supply of CO_2-free air. The exhaust air was passed through a series of CO_2 traps containing a barium hydroxide ($Ba(OH)_2$) solution. The entire content of all traps was titrated with acid to determine the quantity of CO_2 produced.

By the end of the test, cypermethrin cis:trans/40:60 did not show any evidence of biodegradation. It was noted that CO_2 production was lower in cultures containing the test substance than in control cultures. This shows that the test substance had a slightly toxic effect on the activated sludge microbes used to inoculate the cultures. This is also supported by a slight suppression of biodegradation in the toxicity control group.

Degradation of the reference substance, sodium benzoate, exceeded 60% on day 6, and was 89% at the end of the test. These data show that the inoculum was viable and exerting normal degradative activity.

In conclusion, cypermethrin cis:trans/40:60 is **not inherently biodegradable**.

Ultimate biodegradability

In a third test to assess the biodegradation of cypermethrin cis/trans +/- 40/ 60, *Barnes*, 2005 tested the ultimate anaerobic biodegradation of the molecule by measurement of biogas production.

The anaerobic biodegradability of cypermethrin cis:trans/40:60 was assessed using recommendations of *the British Standard (BS) method 6068*, (1996) and International Organisation for Standardisation (ISO) method 11734 (1995" Water quality- Section 5.21; Evaluation of the 'ultimate' biodegradability of organic compounds in digested sludge-Method by measurement of the biogas production). The test cultures contained cypermethrin cis:trans/40:60 (nominally, 20 mg as carbon [C]/L), mineral salts medium (MSM) and pre-digested anaerobic sludge inoculum (solids content, 2.31 g/L), obtained from a plant treating domestic waste water. The potential inhibition of the inoculum by cypermethrin cis:trans/40:60 at the test concentration was assessed in an inhibition assay which assessed biogas evolution from cultures containing the test and reference substances (Polyethylene glycol (PEG 400 AR grade product number P/3676/08, batch 0255601) Fisher Scientific UK). No radio labelled items was used during the study.

The cultures were prepared and handled during the test using bench-top, anaerobic gassing techniques and incubated in darkness at 35 ± 2°C for 60 days.

At the start of the test, the pH of one replicate of the controls, test and inhibition assay series of cultures was determined and the culture discarded. Five replicates of each culture series were incubated and biogas evolution was determined at intervals during the test using a handheld pressure meter. After 60 days of incubation, the pH of each mixture was determined and the inorganic carbon (IC) content of the settled culture medium was measured to provide an estimate of total mineralisation of the test and reference substances.

Biodegradation of cypermethrin cis:trans/40:60 occurs and achieved a mean total level equivalent to 17% by the end of the test on day 60. Nevertheless due to the lack of radiolabelled test item to properly monitor the degradation of the active, RMS consider that the ultimate biodegradability is not properly evaluated and therefore, the 17% degradation value should only be considered as indicative. Substances are considered to be ultimately biodegradable in this test if level of biodegradation achieves 60% of the theoretical level by the end of the test.

In conclusion, anaerobic degradation of cypermethrin cis:trans/40:60 occurs but due to the lack of radiolabeled monitoring of the degradation, the provided value of 17% degradation is only indicative and the test item seems **not to be ultimately biodegradable** under the test conditions.

Water/sediment degradation

Table 385: Water sediment degradation study on cypermethrin cis/trans +/- 40/ 60 (*Brice*, 2006a)

Guideline/ Test method	Test type	Inoculum		Test substance concentration.	Degradation		Reference
		Type	Adaptation		Incubation period	Degree (%)	
OECD 308 GLP = Yes Rel =1	Water-sediment from two sites: Site A; sediment was pH 7.4 Swiss Lake; sediment pH 6.1 Site A; water was pH 8.22 Swiss Lake; water was pH 5.85	N/A	N/A	30 µg/L	100days	After 100 days, the DT_{90} in both systems had been exceeded and all known metabolites that individually comprised > 5% of applied radioactivity were in decline. An unidentified metabolite was still increasing after 100 days. Cypermethrin degraded very rapidly in both water-sediment systems. DT_{50} values were between 3.5 (SFO Kinetic) and 9.8 days in each total sediment system. Dissipation from the water phase was more rapid than from the system as a whole (DT_{50} values were 0.5 days for both systems). Dissipation from the sediment phase was characterized by DT_{50} comprised between 10.9-14.3 days. The metabolites of cypermethrin degraded extensively by mineralization to carbon dioxide. Mean levels of	*Brice*, 2006a, Covance report 1669/014,

						carbon dioxide evolved were over the range 25-69% of applied radioactivity at 100 days. Dissolved carbon dioxide was also present in the water and sediment phases.	

Brice, (2006a) studied the rate of degradation of (^{14}C)-cypermethrin (cis:trans 40:60) in two water-sediment systems over a period of 100 days, according to OECD guideline 308. The application rate was 4.3 µg per unit (water surface area of 15.9 cm^2).

Samples of the 2 mm sieved sediment (3 cm depth in a 4.5 cm internal diameter vessel) and 0.2 mm sieved water (9 cm above sediment) were dispensed into individual glass vessels through which moistened air was drawn. The units were maintained in the dark at 20 ± 2°C for 25 days to enable equilibrium to be established. After treatment with radiolabelled test substance, the air drawn over the surface of the units was passed through a series of traps (ethanediol, 2% paraffin in xylene and two 2M sodium hydroxide solution traps) to collect evolved radiolabelled material.

Dosing was carried out by drop wise application of the radiolabelled test substance (4.3 µg, 21.22 kBq for phenoxy label; 4.3 µg, 20.34 kBq for cyclopropyl label), in acetonitrile (92 µL or 90 µL for the phenoxy and cyclopropyl labels respectively) to the surface water of each water-sediment system. The water-sediment units were incubated in the dark at 20 ± 2°C.

The water was separated from the sediment by aspiration and the two phases were separately analyzed. Non-radiolabelled test substance and potential degradation products were co-chromatographed by high performance liquid chromatography (HPLC) with reference standards. The identities of metabolites were confirmed by thin layer chromatography (TLC) of selected samples using authentic reference standards. Carbon dioxide was confirmed by precipitation of radioactivity from sodium hydroxide solution traps following the addition of barium chloride solution.

The percent of applied radioactivity present as test substance was plotted against the incubation time in days. A curve was constructed through the data points using non-linear regression analysis to give a line of best fit.

The equation used for the surface water and total system was a single-phase exponential model (i.e. first order kinetics):

$$y = C_o \times e^{-kt}$$

Where y is the percent of test substance at time t days and C_0 is the computed initial concentration and k is the rate constant (slope). DT_{50}, DT_{75} and DT_{90} values were calculated from the equation of the lines. They were calculated as the value of t which gave a value of y equal to 50% (DT_{50}), 25% (DT_{75}) and 10% (DT_{90}) of the calculated initial concentration (intercept on the y-axis).

A different equation was used for the sediment as the level of cypermethrin accumulated before it decreased:

$$y = (b \times e^{-k_1 t}) - (a \times e^{-k_a t})$$

Where y is the percent of cypermethrin at time t days and a and b are constants. DT_{50}, DT_{75} and DT_{90} values were calculated as the time taken to decrease from the maximum computed concentration to half, 25% and to 10% of the maximum computed concentration, respectively.

Mean recoveries of radioactivity for both the [^{14}C-phenoxy] cypermethrin and [^{14}C-cyclopropyl] cypermethrin were $\geq$ 90% at each sampling interval, with one exception of 89% at 100 days for dose group D (cyclopropyl label, Swiss Lake).

Dissipation of cypermethrin was effective in both water-sediment systems. DT_{50} values ranged between 3.5 (SFO kinetic otherwise 2.5) and 9.8 days in sediment and 0.5 days in the water phase at 20°C which correspond to 4.7 and 18.5 days in sediment and 0.95 days in water at 12°C. Trans-cypermethrin dissipates faster than the cis-cypermethrin isomer with DT_{50} ranging from 1.1 to 2.9 d

at 20°C for the trans-isomers and 12.5 to 16.9 d for the cis-isomer which correspond to 2.1 to 5.5 d and 23.7 to 32 d at 12°C respectively for cis- and trans- isomers in total system.

The significant metabolites/degradation products were 3-phenoxybenzoic acid (from the phenoxy label) which accounted for 21% in water and 11% in sediment at day 10 in site A and 12% in water and 29% in sediment in swiss lake at day 29; TDCVC which accounted for 38% in water and 20% in sediment in site A at day 58 and 44% in water and 16% in sediment in swiss lake site at day 58; and CDCVC (from the cyclopropyl label) which accounted for 15% in both surface water and sediment at day 29 and day 58 in site A and 22% in water at day 29 and 9% in sediment at day 58. The two metabolites TDCVC and CDCVC are persistent with DT_{50} values > 40 days. A further unknown metabolite (Unknown 1) was identified at levels > 10% in the units dosed with the cyclopropyl label. Despite extensive development of sample clean up and chromatography methods, it was not possible to identify metabolite Unknown 1 within this study. This unknown metabolite is thought to be related to TDCVC, which could be detected in the 10-100 ng range when spiked into the extracts containing the unknown. In both systems there were no other single unidentified metabolites which individually comprised 5% of applied radioactivity at any time point. Also overall radioactivity decreased with time, probably due to loss as radiolabelled carbon dioxide to the atmosphere.

Applied radioactivity unextracted from sediment increased from < 1% at day 0, to maximum values of 18 and 19% at 100 days following application of [^{14}C-phenoxy] cypermethrin and [^{14}C-cyclopropyl] cypermethrin, respectively. Bound residue analysis showed radioactivity was distributed evenly across each fraction.

Table 396: Bound residue in water sediment study (Brice 2006a)

System	Label		Time (days)	Unextracted radioactivity	Bound reside extract	Fulvic acid	Humic acid	Humin
Site A	Phenoxy		45	20.7	11.7	5.7	5.5	8.4
Swiss Lake	Phenoxy		10	20.0	15.0	6.3	8.0	4.9
Site A	Cyclopropyl		29	6.7	4.3	3.2	1.1	2.1
Swiss Lake	Cyclopropyl		100	18.8	14.6	11.0	4.0	3.3

Conclusion: since the mother molecule and both labeled cycles were degraded in both the water and the sediment phase with DT_{50} ranging between 4.7 (SFO kinetic) and 18.5 days in sediment (12°C) and 0.95 day in water, we can conclude that cypermethrin is biodegradable in a water/sediment compartment. However, the two main degradation products TDCVC and CDCVC have to be considered as persistent with typical DT_{50} values > 40 days.

Aerobic degradation in soil

Table 47: Aerobic degradation in soil (*Brice*, 2006c)

Guideline (method used)	Soil type	Application rate	Min % applied radioactivity recovered at end of the study			Max % Applied radioactivity recovered (day)					Reference
			Label	Isomer (cis/trans)	Total Cyp	CO_2	NER	3-PBA	TDCVC	CDCVC	
OECD 307 GLP = Yes Rel =1	¤PT102 (sandy loam)	15µg/50g dry weight equiv soil	Ph*	6.1/2.9	9.0	48.6 (90)	36.8 (90)	7.4 (3)	NA	NA	Brice A and Cooke C., 2006c, Covance study report 1669/012.
			Cy*	7.7/3.4	11.1	70.3 (90)	13.8 (20)	NA	11.9 (7)	2.3 (7)	
	¤PT103 (sandy loam)	15µg/50g dry weight equiv soil	Ph*	13.4/6.8	20.2	39.5 (58)	24.5 (58)	2.4 (7)	NA	NA	
			Cy*	8.4/3.8	12.2	56.3 (120)	16.2 (120)	NA	3.8 (7)	0.6 (7-30)	
	¤SK920191 (clay loam)	15µg/50g dry weight equiv soil	Ph*	3.1/1.8	4.9	53.7 (90)	36.8 (30)	10.2 (7)	NA	NA	
			Cy*	3.4/2.3	5.7	77.8 (90)	17.6 (14)	NA	13.6 (7)	3.9 (7)	
	¤SK15556090 (Silty clay loam/clay loam)	15µg/50g dry weight equiv soil	Ph*	4.4/1.7	6.0	54.2 (90)	34.9 (30)	5.5 (3)	NA	NA	
			Cy*	4.9/2.4	7.3	75.2 (90)	17.0 (30)	NA	7.5 (3-7)	1.8 (7)	
	↑PT102 (sandy loam)	15µg/50g dry weight equiv soil	Ph*	11.6/6.2	18.2	34.6 (120)	26.7 (120)	7.3 (14)	NA	NA	
			Cy*	13.8/7.4	21.2	49.3 (120)	14.1 (120)	NA	12.3 (30)	2.9 (90)	

¤ 20± 2°C ; ↑10±2°C ; Ph* = phenoxy label ; Cy* cyclopropyl label; NER: non- extractable residue

The route and rate of degradation of cypermethrin cis/trans +/- 40/ 60 was studied in one soil type (soil PT102) at 20 ± 2°C by *Brice A. and Cooke C.*, (2006c). The rate of degradation of cypermethrin cis:trans/40:60 was also studied in this soil at 10 ± 2°C, and in three other soil types at 20 ± 2°C. The application rate was 15 µg/50 g dry weight equivalent of soil. This was based on the agricultural application rate of 0.15 Kg/ha (calculated using a depth of 5 cm and assuming a bulk density of 1.0 g/cm^3).

Samples of the sieved soil (50 g) were dispensed into individual glass vessels through which moistened air was drawn. The units were maintained in the dark at 20 ± 2°C or 10 ± 2°C for four days to enable equilibrium to be established. The air drawn over the surface of the units was passed through a series of traps (sodium hydroxide, ethanediol and paraffin in xylene) to collect evolved radiolabelled material.

Dosing was carried out by drop wise application of the radiolabelled test substance (*ca* 15 µg, 73.3 kBq for the phenoxy label and *ca* 15 µg, 71.3 kBq for the cyclopropyl label), in acetonitrile (90 µL for the phenoxy label and 92 µL for the cyclopropyl label) to the soil samples. The soil was mixed thoroughly before incubation in the dark at 20 ± 2°C or 10 ± 2°C.

The soil extracts were co-chromatographed by high performance liquid chromatography (HPLC) with reference standards. The identities of metabolites were confirmed by thin layer chromatography (TLC) of selected samples using authentic reference standards.

The degradation rate of cypermethrin cis:trans/40:60was determined in each soil using a single phase first-order model.

Following application with [^{14}C-phenoxy]cypermethrin, one significant metabolite, 3-phenoxybenzoic acid was present in each soil with a maximum level of 10.2% total applied radioactivity at day 7.

Primary extracts contained 94 to 100% of applied radioactivity immediately after dosing, decreasing to 6 to 35% after 120 days. Volatile radioactivity (carbon dioxide) increased to 49 to 78% of applied radioactivity at the terminal time point. $^{14}CO_2$ confirmatory analysis was performed on the pooled sodium hydroxide traps from each terminal incubation unit, by barium chloride precipitation. No radioactivity was detected in any of the supernatant traps, confirming the presence of carbon dioxide only. Non extractable residues accumulate until the end of the study. The accumulation patterns tends to show a plateau which is usually reach after 30days. The maximum NER founds varies from 13.8% to 36.8% of max applied radioactivity.

Following application with [^{14}C-cyclopropyl]cypermethrin there were two significant metabolites, (1RS)-cis-3-(2, 2-dichlorovinyl)- 2,2-dimethylcyclopropanecarboxylic acid (CDCVC) and (1RS)-trans-3-(2, 2-dichlorovinyl)- 2,2-dimethylcyclopropanecarboxylic acid (TDCVC) present in each soil. The maximum levels of CDCVC and TDCVC were 3.9 and 13.6% total applied radioactivity, respectively at day 7.

Table 408: Degradation rates for cypermethrin metabolites at 20°C (*Brice,* 2006c)

Soil	Label	Compound	Model	Degradation rate (days)		
				DT-50	DT-90	R^2
PT102	Phenoxy	3-Phenoxybenzoic acid	One phase	13.2	41.7	0.987
PT103	Phenoxy	3-Phenoxybenzoic acid	One phase	119.8[2]	391.8[2]	0.839
SK920191	Phenoxy	3-Phenoxybenzoic acid	One phase	9.9	26.7	0.998
SK15556090	Phenoxy	3-Phenoxybenzoic acid	One phase	9.7	31.9	0.969
PT102[1]	Phenoxy	3-Phenoxybenzoic acid	One phase	35.0	116.2	0.954
PT102	Cyclopropyl	TDCVC	One phase	17.6	51.6	0.970
PT103	Cyclopropyl	TDCVC	One phase	35.8	113.1	0.967
SK920191	Cyclopropyl	TDCVC	One phase	10.3	24.0	0.984
SK15556090	Cyclopropyl	TDCVC	One phase	8.9	22.9	0.997
PT102[1]	Cyclopropyl	TDCVC	One phase	65.6	182.5[2]	0.998

PT102	Cyclopropyl	CDCVC	One phase	34.3	114.0	0.975
PT103	Cyclopropyl	CDCVC	One phase	357.2[2]	1186.7[2]	0.754
SK920191	Cyclopropyl	CDCVC	One phase	14.6	48.4	0.966
SK15556090	Cyclopropyl	CDCVC	One phase	12.2	40.5	1.000
PT102[1]	Cyclopropyl	CDCVC	One phase	133.9[2]	444.7[2]	0.519

[1] Incubation at 10 ± 2°C [2] Extrapolated values

The DT_{50} values for the degradation of total cypermethrin cis/trans +/- 40/ 60 in the four soils was 13.9, 24.2, 6.4 and 8.4 days following incubation at 20 ± 2°C. In soil PT 102, incubated at 10 ± 2°C, the DT_{50} value for the degradation of cypermethrin cis/trans +/- 40/ 60 was 52 days. Corrections made to 12°C give DT_{50} values ranging from 12.13 to 45.9 days. The three degradation products 3-PBA, CDCVC, TDCVC, are characterized by DT_{50} of 9.7 to 13.2 days; 8.9 to 35.8 day and 12.2 to 34.3 day respectively at 20°C. Corrections to 12°C provide DT_{50} of 18.4 to 25.0 days, 16.9 to 33.4 days and 23.13 to 65.0 days, respectively. To this regards, the results from the soil PT03 were not considered appropriate due to the fact that they were mainly extrapolated and since they provide results which are not homogeneous with the rest of the data.

The geometrical mean DT_{50} in soil is therefore 20.52 days (3PBA) ; 22.24 days (TDCVC) and 34.66 days (CDCVC) at 12°C for the three metabolite and 17.2 days at 12°C for total cypermethrincis/trans +/- 40/ 60. The arithmetical mean DT_{50} at 20°C is 13.45d which correspond to 25d à 12°C.

Table 419: Degradation rate of total cypermethrin cis/trans +/- 40/ 60 at 20°C (*Brice*, 2006c)

Soil	Label	Model	Degradation rate of cypermethrin (days)			
			DT-50	DT-75	DT-90	R^2
PT102	Both	One phase	13.9	27.8	46.2	0.947
PT102	Phenoxy	One phase	13.0	26.1	43.3	0.947
PT102	Cyclopropyl	One phase	14.8	29.7	49.3	0.956
PT103	Both	One phase	24.2	48.3	80.3	0.934
SK920191	Both	One phase	6.4	12.9	21.4	0.974
SK15556090	Both	One phase	8.4	16.7	27.7	0.968
PT102[1]	Both	One phase	51.9	103.7	172.2[2]	0.975

[1] Incubation at 10 ± 2°C [2] Extrapolated value

Table 50: Degradation rates for cis and trans-cypermethrin at 20°C (*Brice,* 2006c)

Soil	Label	Compound	Model	Degradation rate (days)			
				DT-50	DT-75	DT-90	R^2
PT102	Both	*cis-cypermethrin*	One phase	25.0	49.9	82.9	0.948
PT102	Phenoxy	*cis-cypermethrin*	One phase	22.7	NA	75.4	0.950
PT102	Cyclopropyl	*cis-cypermethrin*	One phase	27.4	NA	90.9	0.957
PT103	Both	*cis-cypermethrin*	One phase	46.6	NA	154.9[2]	0.864
SK920191	Both	*cis-cypermethrin*	One phase	11.0	NA	36.4	0.974
SK15556090	Both	*cis-cypermethrin*	One phase	15.0	NA	49.7	0.975
PT102[1]	Both	*cis-cypermethrin*	One phase	81.5	NA	270.6[2]	0.976
PT102	Both	*trans*-cypermethrin	One phase	8.3	16.6	27.5	0.965
PT102	Phenoxy	*trans*-cypermethrin	One phase	7.8	NA	26.0	0.957
PT102	Cyclopropyl	*trans*-cypermethrin	One phase	8.7	NA	29.0	0.976
PT103	Both	*trans*-cypermethrin	One phase	15.8	NA	52.4	0.974
SK920191	Both	*trans*-cypermethrin	One phase	3.9	NA	19.0	0.997
SK15556090	Both	*trans*-cypermethrin	One phase	5.0	NA	16.7	0.977
PT102[1]	Both	*trans*-cypermethrin	One phase	34.9	NA	116.0[2]	0.966

[1] Incubation at 10 ± 2°C [2] Extrapolated values NA = Not Applicable

The degradation of the cis and trans isomers shows that DT_{50} for the cis-isomer is each time greater that the respective DT_{50} of the trans-isomer, in all soils , whatever the temperature is or whereever on which cycle the label is (See table 48).

In conclusion, **cypermethrin** cis:trans/40:60 was metabolized to three significant metabolites in soil, 3-phenoxybenzoic acid, CDCVC and TDCVC. Cis-cypermethrin degrades at lower rates in comparison to trans-cypermethrin. Further metabolism of cypermethrin and/or these metabolites lead to bound residues and mineralization to carbon dioxide.

Anaerobic degradation in soil

The route and rate of cypermethrin cis:trans/40:60 was studied in one soil type under anaerobic conditions (incubation in the dark at 20 ±2ºC) according to OECD guideline 307 using an application rate of 0.15 Kg/ha (*Brice*, 2006d). Anaerobic conditions were maintained for 182 days with duplicate samples removed for analysis 0, 10, 24, 42, 69, 130 and 192 days after application. The water and soil phases were separated and analysed separately by HPLC and TLC to determine levels of cypermethrin and its metabolites. For the determination of DT_{50}, DT_{75} and DT_{90}, the percent of applied radioactivity present as test substance was plotted against the incubation time in days. Curves were constructed throught appropriate data points using non –linear regression analysis to give lines of best fit. The equation used for curve fitting was a single-phase exponential model (I.e. first order kinetics). DT_{50}; DT_{75}; and DT_{90} were calculated from the equations of the lines.

Table 51: Degradation rate of cypermethrincis/trans +/- 40/ 60 and cypermthrin isomers (*Brice*, 2006d)

Guideline (method used)	Label	Model	Compound	Rate of degradation of cypermethrin (days, 20°C)			References
				DT-50	DT-75	DT-90	
OECD 307 (April 2002) GLP = Yes Rel =1							*Brice, A., Cooke, C.* (2006d); Covance Laboratories Limited, report no. 1669/013-D2149.
	Phenoxy	One phase	Total cypermthrin	46	92	153	
	Cyclopropyl	One phase	Total cypermethrin	46	92	152	
	Phenoxy	One phase	Cis-cypermthrin	58	115	191	
	Phenoxy	One phase	Trans-cypermthrin	31	63	104	
	Cyclopropyl	One phase	Cis-cypermthrin	55	111	184	
	Cyclopropyl	One phase	Trans-cypermthrin	34	68	113	

Table 52: Recovery rate of applied radioactivity on cypermethrin cis/trans +/- 40/ 60 and cypermethrin-isomers respective to metabolites occurrence

Guideline (method used)	Fraction	Min % applied radioactivity recovered at end of the study			Max % Applied radioactivity recovered (day of occurance)						Reference
		Label	Isomer (cis/ trans)	Total Cyp	CO_2	NER	3-PBA	3-PBAD	TDCVC	CDCVC	
OECD 307 GLP = Yes Rel = 1	Surface water	Ph*	ND/ND	ND	ND	NA	16.6 (120)	ND	NA	NA	*Brice A, Cooke, C.*, (2006d), Covance study report 1669/013-D2149.
		Cy*	ND/ND	ND	ND	NA	NA	ND	21.3(120)	15.3 (192)	
	Soil	Ph*	3.5/1.7	5.3	ND	25.1	18.5 (120)	0.7(42)	NA	NA	
		Cy*	4.3/1.9	6.2	ND	9.1	NA	NA	9.9 (120)	7.6 (192)	
	Total system	Ph*	3.5/1.7	5.3	27.3(192)	25.1	35.1	0.7(42)	NA	NA	
		Cy*	4.3/1.7	6.2	22.8(42)	9.1	NA	NA	31.2 (120)	22.8 (192)	

Cypermethrincis/trans +/- 40/ 60 was metabolized to three extractable metabolites 3PBA, CDCVC, TDCVC and carbon dioxide in the total flooded soil system. Their maximum levels were 35.1, 22.8, 31.2 and 22.8% of applied radioactivity, respectively. A fourth metabolite was identified but the maximum level of applied radioactivity for this compound stay below 1%. Further metabolism of cypermethrin and/or these metabolites resulted in bound residue which accounted to max 25.1% and 9.1% of the initially applied radioactivity fort the phenoxy- and the cyclopropyl- cycle respectively and mineralization to carbon dioxide.

Table 423: Reflux extract and bound residues (*Brice*, 2006d)

Soil, Label	Incubation unit	Percent applied radioactivity (%)			
		Reflux extract	Fulvic Acid	Humic Acid	Humin
PT 102, phenoxy	A15 (183d)	8.2	3.9	7.0	5.7
PT 102, cyclopropyl	B8 (32d)	3.3	2.1	1.9	1.9

The distribution of bound residues in the different soil fractions is indicated in the table 51.

Throughout the duration of this study, no cypermethrin was detected in the surface water of the flooded soil system following application with [^{14}C]-cypermethrin. The DT_{50} of total cypermethrin was estimated to 46 days at 20°C. The DT_{50} of the isomers for both labels were 58d, 31d, 55d, 34d for the phenoxy cis and trans isomer and the cyclopropyl cis and trans isomers respectively at 20°C. Normalization to 12°C resulted in DT_{50} of 87.2d for total cypermethrin; 110 d and 58.8 d for the phnoxy cis and trans isomer and 104d and 64.5 d for the cyclopropyl cis and trans isomers respectively.

In conclusion, cypermethrin was metabolized to three significant metabolites in soil under anaerobic condition, 3-phenoxybenzoic acid, CDCVC and TDCVC. Cis-cypermethrin degrades at lower rates in comparison to trans-cypermethrin. Further metabolism of cypermethrin and/or metabolites lead to bound residues and mineralization to carbon dioxide.

5.1.3 Summary and discussion of degradation

The longest half-life determined within the pH ranges 4-9 for cypermethrin cis:trans/40:60 is > 1year at 50°C and > 29d at neutral pH at 25°C. Correction of the half- life calculated from the dark controls of the photolysis study to 12°C results in 98.8 d for pH 7 and 1.65 d at pH 9 resp., whereby the longest half-life doesn't meet the CLP criterion of < 16d.

Cypermethrin cis/trans +/- 40/ 60 is photolysed in water (68% AR of total recovery of test substance, quantum yield = 0.0308), in the air (estimated half-life of 0.75d) and in soil (calculated half-life of 3d).

Cypermethrin cis/trans +/- 40/ 60 is not ready biodegradable (0.6-1.4% after 33d), not inherently biodegradable nor ultimately biodegradable. However, cypermethrin cis/trans +/- 40/ 60 is degradable in a water-sediment compartment (DT_{50}whole system between 3.5 and 9.8d) and ultimate degradation ranged between 25-69% of AR at 100d. 3 major (non-classified as toxic) metabolites are formed: 3PBA, CDCVC, TDCVC. CDVC and TDCVC are persistent with a DT_{50} > 40d.

Under aerobic condition, arithmetical mean DT_{50} in soil at 12°C is 25d. Cypermethrin cis/trans +/- 40/ 60 is metabolized in three metabolites, 3PBA, CDCVC, TDCVC, and CO_2. Under anaerobic condition, the normalized DT_{50} to 12°C was 87.2d. The substance is also metabolized in the same three metabolites and CO_2.

Cypermethrin cis/trans +/- 40/ 60 is considered not rapidly degradable in comparison with the classification criteria for degradation.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

The adsorption and desorption properties of cypermethrin cis:trans/40:60 were evaluated by *Brice*, (2006b). The adsorption characteristics of [^{14}C]-cypermethrin were determined in four soil types and one sediment (Table 53) according to OECD Guideline 106 (January 2000).

Summary

Table 434: Summary of adsorption/desorption study with cypermethrin cis/trans +/- 40/ 60

Guideline/ Test method	Temperature (ºC)	Radioactive labelling	Test substance conc. (µg/L)	Soil	Material balance	Reference
OECD 106 GLP = Yes Rel = 1	20±2°C	Yes. [^{14}C -phenoxy]-cypermethrin cis and trans isomers.	2.0µg/ml	5 types see table 53	Soil = 0.6-1.3% Supernatant = 96.8-101.1%.	*Brice*, 2005, covance study 1669/015

Table 445: Soil and sediment characteristics (Brice, 2006b)

Soil type	Sand (%)	Silt (%)	Clay (%)	Organic C (%)	pH	Reference
EL-7 (clay loam)	31	45	24	5.2	6.3	*Brice* 2006b, covance study 1669/015
SK566696 (loamy sand)	84	5	11	1.4	4.2	
SK961089 (clay loam)	36	36	28	8.3	7.5	
Matanuska (sandy silt loam)	28	63	9	5.5	4.7	
Site C1 sediment	76	15	9	2.9	5.4	

Soil samples (0.5 g dry weight equivalent) were pre-equilibrated with 0.01 M calcium chloride solution (25 mL) overnight. They were then treated with solutions of [^{14}C]-cypermethrin prepared in acetonitrile (20 µL) to produce duplicate samples per soil, with an initial nominal concentration in the aqueous phase of 2.0 µg/mL. Recovery of applied radioactivity was determined by radioassay of the adsorption supernatants and remaining soil/sediment. In an attempt to reduce the degradation of cypermethrin cis/trans +/- 40/ 60, tests were repeated in the dark at 20 ± 2°C under sterile conditions.

The initial ratio of soil to aqueous phase test under non-sterile conditions demonstrated that only very low levels of radioactivity were present in the supernatant. This test also demonstrated that only trace levels of the radioactivity in the supernatant corresponded to cypermethrin.

Due to the relatively low water solubility of cypermethrin cis/trans +/- 40/ 60 and its instability in the test system, it was not possible to determine adsorption and desorption isotherms for this compound. Freundlich adsorption coefficient (K) values could not therefore be determined. In order to make an estimated assessment of the mobility of cypermethrin the **minimum K_{oc}** values of this compound in each soil/sediment were used (see table below).

Table 456: Estimated Koc (*Brice*, 2006b)

Soil type	Koc	*Kd	Reference
EL-7 (clay loam)	≥202418	-4858	*Brice* 2006b, covance study 1669/015
SK566696 (loamy sand)	≥574360	-4595	
SK961089 (clay loam)	≥80653	-3871	
Matanuska (sandy silt loam)	≥152388	-4876	
Site C1 sediment	≥527972	-8976	
Accoding to Qsar 1 (log pow 5.3-5.6)	2,676,77– - 4,586,002	/	

According to the Qsar in the TGD on Risk Assessment, part III ($\log K_{oc} = 0.81 \log K_{ow} + 0.10$)) the value for K_{oc} is 2,676,776 and 4,586,002 for Log P_{ow} 5.3 to 5.6, which confirms that the result of the test only provide low values for K_{oc}.

Conclusion: These results indicate that the minimum K_{oc} values ranged from 80653 to 574360 in the four soils and is minimum 527972 in the sediment.

K_{oc}= 575 x10E03

Cypermethrine cis/trans +/- 40/ 60 has a high potential to adsorp (immobile according to *Mensink et al.*, 1995).

5.2.2 Volatilisation

Cypermethrin cis/trans +/- 40/ 60 is characterized by a vapour pressure of 2.3×10^{-7} Pa at 25°C according to *Sidney* (2005) (GLP, Rel = 1).

Bates, (2002a), (GLP, Rel = 1) calculated the Henry's Law Constant of cypermethrin to be 0.024 $Pa.m^3.mol^{-1}$ at 20°C indicating that is unlikely that there will be significant partition from the aqueous environment to the air.
This value was calculated using :
Vapour pressure at 20°C = 2.3×10^{-7} Pa

Water solubility at 20°C 4 µg/L = 9.6 x 10^{-6} mol/m^3

5.3 Aquatic Bioaccumulation

Table 467: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
Partition coefficient octanol/water	Log P_{ow} = 5.3-5.6	/	Bates 2002a
OCDE 305E	373.4 (±45.35) L/Kg_{ww}		G.Szeleczky,1990
BFCwin; EPISUITE	BCF= 417 L/Kg_{ww}	QSAR	RMS

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

The Technical Guidance Document on Risk Assessment (TGD) provides an equation allowing for an estimation of the BCF based on the Log K_{ow}. The calculated BCF value according to equation log BCF fish = 0.85 • Log K_{ow} − 0.70 (TGD on Risk Assessment, part II, equation n°74) is 11481.53 L/Kg_{ww} for Log K_{ow} = 5.6.

Another calcultation can be made by the mean of the QSAR BCFwin from the tool EPISUITE to calculate a BCF for cypermethrin. This Qsar is deemed to be more relevant for pyrethroids since correction factor for large molecule is included in the algorithm. The results proposed by this Qsar is much more closer to results of laboratory tests where both are available and reliable (see below).

Using BCFwin (EPISUITE) for a Log P_{OW} of 5.45, a Log BCF of 2.62 is calculated which correspond to a BCF of 417 L/Kg $_{ww}$ (Data-Base Structure Match: Cypermethrin) The Log BCF calculated based on the Log P_{ow} with correction for cyclopropyl esther (-1.259) is 1.733 which correspond to a BCF of 54.11L/Kg_{ww}.

5.3.1.2 Measured bioaccumulation data

Table 478: Measured bioaccumulation data

Guideline/ Test method	Species.	Test material conc. (mg/L)	Radio-labelled	Exposure		BCF	Elimination	Remarks	Reference
				design	duration				
OECD guideline (1981) part 305E GLP = Unclear, but very well documented Rel =1	Rainbow trout	0.1ppb	No	Flow-through	10 days	373±45	Depuration rate constant = 0.00158 - 1/hr	Not bioaccumulative	[redacted], *Chimac-Agriphar* (1990), 90-016 CYP/T133, 1990

[redacted]. (1990) has evaluated the bioaccumulation potential on fish of cypermethrin cis:trans / 40:60 (purity 94.2%) according to the OECD 305E protocol. A flow-through system was employed with a test substance concentration of 0.1 ppb. A solvent was added to enhance the solubility of cypermethrin.

The uptake phase lasted for 10 days and the depuration phase was 20 days. Unchanged test substance in both the aquaria water and whole fish homogenate was determined by GC-ECD. The determination of unchanged test substance level in fish homogenate indicated that the concentration elevated rapidly until the 160th hour and further but small increase could be observed till the end of the uptake phase. In spite of Log P_{ow} of 4.47 which would predict a steady state reachable within 85h, it tooks 240h for cypermethrin to reach a quasi steady state level in the fish. The BCF reached 373.4 (±45.35) by the end of the uptake phase and the depuration rate constant was found to be 0.00158 l/h. The low measured BCF value in comparison to the calculated value (11481.53 with log K_{ow} = 5.6) may be explained by the duration of the uptake phase which was only 10 days and by the addition of the solubiliser.

5.3.2 Summary and discussion of aquatic bioaccumulation

According the measured value for bioaccumulation a BCF value of +/-400 can be estimated. The substance doesn't meet the CLP criterion of BCF≥ 500. It can be concluded that the substance has no potential to bioaccumulate.

5.4 Aquatic toxicity

Table 489: Summary of relevant information on aquatic toxicity

Method	Results	Remarks	Reference

Acute toxicity			
Onchorhynchus mykyss OECD 203 GLP = Yes Rel = 1	96h LC_{50} = 2.83µg/L (geom.mean)	Semi static Sovant = acetone	_[redacted], Covance study 1669/018-D2149
OECD 202; acute tox to *Dapnia magna* 48h GLP = Yes Rel = 1	EC_{50} = 4.7 µg/L	Static Solvant = Acetone	[redacted]Covance report 1669/019-D2149
OECD202 *Daphnia magna* GLP : no Rel = 3 (= not considered relevant)	EC_{50} = 0.3 µg/L	Semi-static Solvant = acetone	Review report/DAR (PPP). *Stephenson*, 1981.
OECD 201; Growth inhibition on algae 96h *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) OECD201 GLP =Yes Rel = 1	EbC_{50} >33.0 µg/L ErC_{50} >33.0 µg/L	Static, limit test Solvant = acetone	[redacted], Covance report 1669/020- D2149
OECD Guideline 202 I *Selenastrum capricornutum* GLP = No, Rel = 3	ErC50 > 100 µg a.s./l	static test Solvant = acetone	*Stephenson*, 1981.
OECD 209 ; Inhibition of microbial activity (aquatic; 3h)	EC50 = 163 mg/L	Activated sludge	*Bealing*, 2002, Covance study no 40/46-D2149

Chronic toxicity			
Pimephales promelas OECD 210 (ELS) Rel = 1	28dph NOEC = 0.463 µg/L (mean meas.) (hatching success) LOEC = 0.463 µg/L	Flow through Key study	[redacted] Covence Laboratories Study number 8252888
Pimephales promelas OECD 210 (ELS) GLP = No Rel = 2	28dph NOEC = 0.03 µg/L	Flow through	[redacted], 1983 Review report/DAR (PPP).
Pimephales promelas OECD 210 (ELS) GLP = No Rel = 2	NOEC=0.25µg/l	Flow through	[redacted],1980 Review report/DAR (PPP).
Pimephales promelas OECD210 (ELS) Rel = 3	28dph NOEL=0.01µg/l (nominal.) (hatching success, fry survival, fry growth) effect considered as biological. Empirical NOEC 0.32 µg/L	Flow through, Study suffers of deficiencies	[redacted]2005, Charles River Laboratories, study number 805972
OECD 202, Part II Reduction in reproduction , *Daphnia magna* 21d GLP = Yes Rel = 1	NOEC 0.04µg/L	Semi-Static	*Dickhaus, S.* , 1990, Pharmatox, Report E.H./B. 2-7-44-90 (CYP/T143)
OECD 211, Reproduction , *Daphnia magna* 21d GLP = Yes Rel = 1	0.053µg/L	Semi-Static	*Simon M.* , 2015,
Pseudokirchneriella subcapitata OECD201 GLP = Yes Rel=1	NOEbC ≥33.0µg/l	Static, limit test	*Manson*, 2005c, Covance report 1669/020- D2149
Mesocosm study			
No: Study conducted to support the agricultural use of cypermethrin, taking into account the recommendations of workshops on higher tier risk assessments for aquatic organisms Multiple species (e.g. HARAP, CLASSIC). GLP = Yes Rel = 1	Min NOAEC= 0.05µg/L	2 applications separated by 14d	*Schnoder, F.*, 2003, Covance Laboratories Ltd, study no. 0040/045 (CYP/T331),

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish (*Manson*, 2005)

Table 60: Short-term toxicity to fish (*Manson*, 2005)

Guideline/ Test method	Spp.	Endpoint	Exposure		Results (µg /L) measured			Reference
			design	duration	LC_{50}	LC_{100}	NOEC	
OECD 203 GLP = Yes Rel = 1	Onchorhynchus mykiss	Death	Semi-static	96 hour	2.83	4.11	n.d.	[redacted],Covance study 1669/018-D2149

n.d.= not determined

From CAR (biocides)

[redacted], (2005) has studied the acute toxicity of cypermethrin cis:trans /40:60 on *Onchorhynchus mykyss* in a 96h semi static design study according to OECD 203 guideline. The definitive test was conducted at nominal exposure concentrations of 0.401, 0.882, 1.94, 4.27, 9.39, 20.7, 45.5 and 100 µg/L, based on the results of two range-finding tests. Temperature, pH, and dissolved oxygenwere monitored for control as wel as for test item during the entire study every 24h and after renewal of the water.

The test item concentrations where monitored for freshly prepared media at 0 and 72h and for old test media at 24 and 96h. Some of the values where outside the 80-120% of the nominal range, therefore the toxicity values were therefore expressed in terms of the geometric mean measured concentrations. LC50 values were calculated using a Probit method.

The 24-hour LC50 toxicity value was calculated to be 4.26 µg/L (95% CI not determined). The highest concentration at which no mortality occurred was 1.94 µg/L. The lowest concentration at which 100% mortality occurred was 8.74 µg/L.

The 48, 72 and 96-hour LC50 toxicity values were all calculated to be 2.83 µg/L (95% CI not determined). The highest concentration at which no mortality occurred was 1.94 µg/L. The lowest concentration at which 100% mortality occurred was 4.11 mg/L.

Conclusion:

Cypermetrin cis:trans/40:60 shows an acute **LC_{50} (96h) of 2.83µg/L** on fish.

5.4.1.2 Long-term toxicity to fish

Table 61: Summary of long-term toxicity studies to fish

Guideline/ Test method	Spp.	Endpoint	Exposure		Results (µg a.s./L) measured			Reference
			design	duration	Effect	NOEC	LOEC	
OECD 210 GLP = Yes Rel = 1	*Pimephales promelas*	Fry survival, Body length, Body weight	Flow through	Embryo-development, hatching and 28 days post hatch	Hatching success ranged from 70 to 90%. In control (mean 80%) and 92 to 97 in tests vessels. No significant effect in hatching success between solvent control and 1.22µg/L nominal. (0.463 µg/l geo-mean measured concentration)	0. 463	LOEC (fry survival) 0.463	[redacted]., 2012 Covence Laboratories Study number 8252888
OECD 210 GLP = No Rel = 2	*Pimephales promelas*	Fry survival, Body length, Body weight	Flow through	Embryo-development, hatching and 30 days post hatch	Hatching success ranged from 95.5 to 98.5%. No significant effect in hatching success between solvent control and 0.5µg/L nominal.	0.25	LOEC (fry survival) 0,5	[redacted]., 1980
OECD 210 GLP = No Rel = 2	*Pimephales promelas*		Flow through	Embryo-development, hatching and 34 days post hatch	Hatching success ranged from 95.5 to 98.5%. No significant effect in hatching success between solvent control and 0.5µg/L nominal.	0.03	LOEC (fry survival) 0.12	[redacted], 1983
OECD 210 GLP = Yes Rel= 3	*Pimephales promelas*	Fry survival, Body length, Body weight	Flow through	Embryo-development, hatching and 28 days post hatch	Hatching success ranged from 31 to 84%. No significant effect in hatching success between solvent control and 1µg/L nominal. All fry at 1µg/L were dead by post-hatch day 11.	0.01 (nomin.) Empirical concl TM 2011) Following RMS NOEC should be 0,32	LOEC (fry survival) 0.9	[redacted]_ 2005, Charles River Laboratories, study number 805972

From CAR (biocides):

Two studies testing the chronic effect of cypermethrin cis:trans/ 40:60 on fish were provided under biocide framework.

[redacted]., (2005) and [redacted]., (2012), have both evaluated the effect of prolonged exposure to Cypermethrin in the cis:trans ratio of 40:60, on the early-life stages of the Fathead Minnow (*Pimephales promelas)* (embryo development, hatching and for 28 days post-hatch), in accordance with OECD Guideline 210 and OPPTS 850.1400 Fish Early-Life Stage Test.

Both tests were conducted under continuous flow through conditions, with embryos (respectively less than 25 h old and 24h on exposure to the test solutions).

In the first study submitted under biocidal framework,([redacted], 2005) larvae/fry were exposed to the following nominal concentrations of [^{14}C]-cypermethrin; 0.01, 0.032, 0.1, 0.32 and 1 µg/L. In the second study ([redacted], 2012), larvae and fry were exposed to non-radiolabeled cypermethrin at the following nominal concentration; 0.005, 0.0015, 0.0045, 0.0135, 0.0405 and 1.22 µg/L. Both solvent and non-solvent controls were included in the tests. Duplicate tanks were tested at each concentration.

[redacted], (2005)

On each day of the study, starting at 48 h prior to the addition of the embryos, single aliquots (10 mL) of water were sampled from each replicate solvent control and test item tank and mixed with scintillation fluid (Zinsser Analytic) prior to determination of radioactive content by Liquid Scintillation Counting (LSC).

The effects were assessed by the mean of the following parameters: Hatching success, fry survival, fry growth (length), and fry growth (weight).

Hatching succes– - results indicated a significantly lower hatching success at 0.1 and 0.32 µg/L nominal, when compared to the control. There was however no statistically significant difference in hatching success between all other groups, including 1 µg/L nominal and the control. As a result, it was not possible to statistically determine a NOEC with respect to hatching success. It is considered by the laboratory that this non-monotonic response does not indicate an effect of the test item but was rather a biological effect. On study day 2, a significant number of dead/fungus-covered embryos were removed from test concentrations of 0.1 and 0.32 µg/L which resulted in a lower hatching success at these test concentrations. This effect is regarded by the authors as a result of a contamination (biological effect) rather to a chemical effect of the molecule. An empirically derived NOEC would be regarded as 0.01 µg/L nominal (0.089 µg/L based on mean measured concentrations) according to the TM 2011.

Fry survival– - results indicated no significant difference in mortality between the 0.01 to 0.32 µg/L treated groups and the solvent control. The NOEC for fry survival is therefore concluded to be 0.32 µg/L nominal (0.30 µg/L mean measured), with the LOEC being 1 µg/L nominal (0.89 µg/L mean measured).

Fry growth (length)– - Body length of surviving fry was found to be significantly greater in all treated groups when compared to the solvent control. This may be explained by the number of fry present in each tank. The solvent control replicates contained significantly more fry than all other tanks thus increasing competition for both food and space, resulting in slightly slower growth. The NOEC for deleterious effects on length was calculated as 0.32 µg/L nominal (0.30 µg/L mean measured) with the LOEC being 1 µg/L.

Fry growth (weight)– - Body weight of surviving fry was found to be significantly greater in all treated groups when compared to the solvent control. This may be explained by the number of fry present in each tank. The solvent control replicates contained significantly more fry than all other tanks thus increasing competition for both food and space, resulting in slightly slower growth. The NOEC for deleterious effects on body weight was calculated as 0.32 µg/L nominal (0.30 µg/L mean measured) with the LOEC being 1 µg/L.

Other effect– - It was observed on study day 4 that all fry at 1 µg/L nominal appeared smaller and weaker than fry in all other tanks. They were observed to be swimming in spiralling movements with periods when they appeared to go into spasms. All fry at 1 µg/L nominal (0.89 µg/L mean measured) were dead by post-hatch day 11.

It was noted on study day 8 that in each replicate tank (with the exception of replicate tank B at 0.32 µg/L nominal) there were numbers of smaller and weaker fry. From study day 21 to the end of the test these smaller and weaker fry began to undergo significant mortality. All mortality from study day 21 to the end on the test was of these smaller and weaker fry.

Comment RMS. Bacterial or fungal infection should generally be considered as evidence for over toxicity if the occurrence or rate of occurrence is greater in exposed tanks than in the controls. However the RMS is of the opinion that in this case the fungal infection is not an evidence of over toxicity. In the *Knight,* (2005) study, dead/fungus-covered embryos were found in some but not all replicate aquaria at concentration 0.1 and 0.32µg/L, and were not seen at all at the upper concentration of 1µg/L. If an effect appears due to overt toxicity, then this effect should be seen in all replicates of a dedicated concentration and also at the higher concentrations. The outcome of the discussion of TM biocide was to set in a conservative approach a NOEC of 0,01µg/L (base on hatching success). According to RMS, this NOEC is too conservative and a NOEC of 0,32µg/L (based on growth rate) is more appropriate. This was also the point of view of industry wich proposed to provide a new ELS test.The latest has been submitted in 2012(Taylor; 2012).

Other reliable long-term fish toxicity studies ([redacted], (1980), [redacted], (1983)), originally submitted in the PPP framework, are available. RMS disregards the study from [redacted], (2005) due to a lack of confidence in the results. RMS allocates a **reliability of 3** to this study.

[redacted], (2012)

Samples of test media from each of the cypermethrin treatments and from the control and solvent control treatments were taken for analysis from individual vessels on day 0 pre-hatch and days 0, 6, 13, 20 and 27 post-hatch. Samples of the concentrated solvent stock solutions were also taken to confirm correct preparation. Sampling of these was performed at day 0 pre-hatch and on days 6 and 27 post-hatch.

Hatching Succes– -Hatching success in the control vessels ranged between 70 - 90% and provided a mean value of 80%. Hatching success in the solvent control group ranged between 90 - 100 % with a mean value of 98%.

Statistically, the hatching success in the control group was significantly lower compared to the solvent control. Hatching success in the control group was well above the validity criterion of 66% therefore this difference is not considered to be biologically significant.

As hatching success in both the control and solvent control groups exceeded 66%, the validity criterion for hatching success was satisfied.

First egg hatch in all treatment, control and solvent control vessels occurred across the ca. 72-hour pre-hatch period. There was no apparent difference in the time to first hatch across any of the treatments when compared to the controls.

There was no treatment-related effect on the hatching success of the embryos. As no significant effects were determined the LOEC could not be accurately defined. In terms of geometric mean measured concentrations the NOEC and LOEC for hatching success were therefore 0.463 and > 0.463 µg/L, respectively.

Survival– - The mean post-hatch survival in the control and solvent control groups was greater than 70% therefore this validity criterion was satisfied. There was no treatment related effect on the post-hatch survival of the fish. As no significant effects were determined the LOEC could not be accurately defined. In terms of geometric mean measured concentrations, the NOEC and LOEC were therefore considered to be 0.463 µg/L and > 0.463 µg/L, respectively.

Fish Total Lengths and Wet Weights- Statistically, the mean length of fish in the control group was significantly lower compared to the solvent control and the wet weight of fish at the lowest treatment concentration (<LOQ) was significantly higher compared to the solvent control. Neither of these differences are considered to be biologically significant as the length and weights were well within the usual ranges for this species.

There was no treatment related effect on the total length or wet weight of the fish. As no treatment related effects were determined the LOEC could not be accurately defined. Therefore, in terms of geometric mean measured concentrations, the NOEC and LOEC were considered to be 0.463 µg/L and > 0.463 µg/L, respectively. No treatment related abnormalities were observed during the conduct of the definitive test.

Conclusion

The first study ([redacted], 2005) suffers of deficiencies which makes the drawing of a conclusion on the effects difficult. Fungal contamination of some replicates was observed and may lead to false results and impede the interpretation of the study. During TM II 2011 it was concluded that the study is not sufficiently robust and in the absence of other results, in a conservative approach, an overall NOEC of 0.01 µg/L measured was established. The RMS does not agree with this NOEC (see comment RMS above).

The study of [redacted], (2012) adresses the point more accurately, suffers no deficiencies and provides a robust NOEC of 0.463 µg/l. As the study of [redacted], (2005) is considered of low reliability, the NOEC from the study of [redacted], (2012) is taken into account for CLH purposes

NOEC Fish = 0.463 µg/L

From DAR (PPP)

In the DAR for cypermethrin, two studies are available: the [redacted], (1980) study (NOEC = 0.25µg a.s./L) and the [redacted], (1983) study (NOEC = 0.03 µg a.s./L, the Review Report endpoint). These studies are not GLP, but are dating before 25/07/1993 (GLP compliance not required for older studies).

[redacted] (1980): The Toxicity of Cypermethrin to Fathead Minnow (*Pimephales promelas*) Embryos and Larvae,

Guidelines : OECD guideline 210

GLP : no

Material and Methods :

Test substance : cypermethrin, chemical purity : 91.5%

Test species : fathead minnow (*Pimephales promelas*)

Number of organisms, age : 60 embryos / concentration, the embryos were obtained within 48 hours after fertilization. 40 organisms used in the 30 day post-hatch part of the study.

Type of test : flow through test

Applied and measured concentrations :

nominal : water control, solvent control, 0.031, 0.062, 0.12, 0.25, 0.50 µg a.s./l

Concentrations were not measured.

Test conditions :

temperature : 25-26 °C,

pH : 6.9-7.4

oxygen content : 8.2-8.4 mg/l

total hardness : 25 mg/l $CaCO_3$

Photoperiod : A 12 hour light 12 hour dark photoperiod was used, with an intensity of 2 - 100 foot-candle.

Analytical methods : -

Findings :

Table 492: Effects on *Pimephales promelas* embryos exposed to cypermethrin

	Concentrations in µg a.s./l						
	solvent control	water control	0.031	0.062	0.12	0.25	0.50
hatchability (%)	95.5	98.5	95.5	98.5	98.5	96.5	97.5
30 day-old larvae - survival (%)	97.5	96.5	92.5	91.0	96.5	91.0	17(*)
- length (mm)	21	20	21.5	21	21	30.5	24.5
- weight (mg)	62.5	58.5	69	68.5	64.5	62	127.5

All results were based on nominal concentrations.

Survival of larvae exposed to 0.5 µg/l was significantly reduced relative to the control (*). No adverse effects on hatchability of embryos or the growth of larvae following exposure to 0.5 µg/l cypermethrin. Thirty day old larvae surviving exposure to 0.5 µg/l cypermethrin were larger than the control (due to reduced competition).

Conclusions :

NOEC (Pimephales promelas 30-day old larvae) = 0.25 µg a.s./l

[redacted], (1983): WL85871 and cypermethrin: A comparative study of their toxicity to the fathead minnow *Pimephales promelas* (Rafinesque).

Study summary:

Guidelines: OECD guideline 210

GLP: No

Material and methods:

Test substance: cypermethrin, chemical purity: 97.5-98.5%

Alpha-cypermethrin, chemical purity: 98.2-99.4%

Test species: fathead minnow (*Pimephales promelas*)

Number of organisms, age: 30 embryos/concentration, the embryos were obtained within 24 hours after fertilization and were followed up to day 34.

Type of test: flow through test

Applied and measured concentrations: Nominal: control, 0.03, 0.1, 0.3, 1.0 µg a.s./L

Recoveries of 97% and 95% respectively for cypermethrin and alpha-cypermethrin. Results are expressed as measured concentrations.

Test conditions:

Temperature: 22.5-25.0 °C

pH: 7.6-8.1

oxygen content: 7.5-9.1 mg/l

total hardness: 230-290 mg/L $CaCO_3$

Photoperiod: a 12 hour light 12 hour dark photoperiod was used

Analytical methods: gas chromatography

Findings:

Table 503: Effects on *Pimephales promelas* embryos exposed to cypermethrin

	concentrations in µg a.s./L (measured)				
	control	<0,03 (*)	0,12	0,17	0,79
pre-hatch mortality (%)(day3)	11	7,5	3,3	7,5	13
post-hatch mortality (%)(day 6)	22	20	17	29	43***
overall mortality (%)(day 34)	38	36	56**	56**	100
weight (mg)(day 34)	78	73	85	78	/

(*) at the limit of determination

Conclusions:

NOEC (*Pimephales promelas* 34-day old larvae, cypermethrin) = 0.03 µg a.s./L

The acute DT_{50} of juvenile fish was determined in an apart experiment:

DT_{50} (*Pimephales promelas* embryos, cypermethrin, 96h) = 1.2 µg a.s./L (confidence limits: 0.55-1.4)

Conclusion PPP:

Both study have been declared acceptable. The study from [redacted], (1983) present the lowest NOEC value of 0.03 µg a.s./L

Overal conclusion Chronic toxicity to fish:

The chronic toxicity to fish has been evaluated in four studies, [redacted], 1980; [redacted], 1983; [redacted], 2005 and [redacted] 2012. The study by [redacted], 2005 is considered as not reliable by RMS and therefore is given a reliability factor of 3. The other studies are deemed acceptable. The state of the art study is the most recent one, ([redacted], 2012). This study provide results comparable with the study of [redacted](1980) with a NOEC ranging between 0.25µg a.s./L and 0.463 µg a.s./L. The study of [redacted] 1983 however provides the lowest accepted NOEC of 0.03µg a.s./L.

NOEC Fish= 0.03µg/L

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Table 64: Short-term toxicity to aquatic invertebrates

Guideline/ Test method	Species	Exposure		Results (µg a.s./L) measured			Reference
		design	duration	EC50	EC100	NOEC	
OECD 202 GLP = Yes Rel= 1	*Daphnia Magna*	Static	48-hr	4.7	Could not be determined.		*Manson*, 2005b, Covance report 1669/019-D2149
GLP = No Rel= 3 (due to lack of recovery of Test item)	*Daphnia spp*	Semi-static	48-hr	0.3	-		*Stephenson*, 1981

From CAR (Biocides)

Manson (2005c) has evaluated the effects of cypermethrin cis:trans/40:60 on *Daphnia magna* according to the OECD 202. Based on the results of the range-finder study, nominal concentrations of cypermethrin in the definitive study were 1.94, 4.27, 9.39, 20.7, 45.5 and 100 µg/L in acetone.

The test item concentrations were measured at the beginning and at the end of the test.

The overall geometric mean measured concentrations of cypermethrin cis:trans/40:60 in samples of test media were 1.05, 2.55, 7.20, 14.2, 32.7 and 56.1µg/L respectively, corresponding to 54.1, 59.7, 76.7, 68.6, 71.9 and 56.1% of the nominal cypermethrin cis:trans 40:60 concentrations respectively. As the mean measured concentrations were outside 80 to 120% of the nominal concentrations, the toxicity values are expressed in terms of mean measured concentrations.The 24 hour EC50 toxicity value was calculated to be outside of the experimental range (971 µg/L) and 95% confidence limits could not be determined. The corresponding no observed effect concentration (NOEC) was 1.05 µg/L (5% immobility occurred at this concentration but is not considered to be biologically significant). The lowest concentration causing 100% immobility at 24 hours could not be determined. The 48-hour EC50 toxicity value was calculated to be 4.71 µg/L (equivalent to 6.87 µg/L nominal), 95% confidence limits could not be determined. The corresponding NOEC and the lowest concentration causing 100% immobility could not be determined.

Conclusion:

Cypermethrin cis:trans/40:60 shows 48h acute effect to *Daphnia magna* with a **48h EC_{50}= 4.71µg/L**. which is close to the water solubility (4µg/L).

From DAR (PPP)

Stephenson, 1981: WL85871 and cypermethrin: A comparison of Their Acute Toxicity to *Salmo gairdneri, Daphnia magna* and *Selenastrum capricornutum.*

Study summary: Guidelines: OECD Guideline 202 I

GLP: No

Material and Methods:

Test substance: cypermethrin, chemical purity: 98.0-98.5%

Test species: *Daphnia magna*

Number of organisms, age: 30 daphnids less than 24 hour old/test group

Type of test: 48 hours semi-static with test solution renewal after 24 hours

Applied and measured concentrations: nominal: acetone control, 0.1, 0.2, 0.5, 1.0, 1.5, 2.5, 4.0, 6.5 and 10 µg/L. The analysis of the test solutions showed that there was a mean decline of 46% and 49% in the concentration of cypermethrin between renewals (24 hours).

Initial measured concentrations were 68-213% and 23-140% of the nominal concentrations for cypermethrin.

Test conditions:

Temperature: 20 ± 2 °C

pH: 8.2 ± 0.3

oxygen content: 9.1 ± 0.4 mg/L

total hardness: 260 ± 20 mg/L as $CaCO_3$

Photoperiod: -.

Analytical methods: gas chromatography

Findings and conclusions:

EC_{50} (cypermethrin, 48h) = 0.3 µg a.s./L (95% confidence limits = 0.2-0.4).

Comment RMS: Acute daphnia endpoint in review report for PPP (EC_{50} = 0.3 µg a.s./L) (study from *Stephenson,* 1981) is not taken into account for CLH purpose. This study is not GLP, and no quality insurance is available which may hide additional deficiencies. The methodology is poorly documented in the report, the guideline used (OECD 202) has been revised to alow less variability in the results and a better reproductibility. The recovery of the substance as regards to nominal concentration is poor and the range of recovery is extended (23% to 140%). Since the concentration in test item is known to decline with time, the timing between sampling and analysis is crucial. The result are expressed based on measured concentration. A low recovery means a low concentration and thus a low apparent effect level. The result of this study is in contradiction with the state of the heart study on short-term toxicity to invertabrates and also with the results of the high reliability long term toxicity studies on invertabrates were no lethal effect on parents were observed at so low concentration for parents after 48h (see 5.4.3).

Furthermore, in the Stephenson study, the identity of the substance as regards to the isomeric composition is, the source of the test item and a batch number is not reported. In the case of cypermethrin it is know that toxicity differs from isomers to isomers. A R configuration at the cyclopropane C-1 position is essential for neurotoxicity; the corresponding 1-S enantiomer is non-toxic. The configuration of the α-cyano group also influences toxicity: a S configuration of the α-cyano carbon is a potent mammalian toxicant, whereas the α-R enantiomers are essentially non-toxic (*Weipung, L. et al*., 2004). *Weipung L. et al,* (2005) has shown that in the case of cypermethrin, these enantiomers contributed for almost all the toxicity to aquatic invertabrates (*Cerodaphnia dubia* or *Daphnia magna*) which confirms the founding made for mammalian toxicology. Increase content of the active enantiomers decreases the LC_{50}. Linear regression of the LC_{50} values against the content of insecticidally active enantiomers showed close correlation (r^2=0.995).

Stephenson compaired in his study the toxicity of Alha-cypermethrin to the toxicity of cypermthrin. Alpha-cypermethrin is composed of two isomer only, one of those is known to be the most toxic. It is not know whether Stephenson has used a cypermethrin with an isomer ratio different from 40:60 in the 1981 study but any change in this ratio may lead to a different picture in toxicity. Since the current document is dedicated to the classification of one specific cypermthrin (CAS n° 52315-07-8; INDEX n° 607-421-00-4) within the different cypermethrins available on the market, **RMS is to the opinion that study performed with an unknown isomeric ratio should not be used for a classification purpose.** Therefore the deficiencies in the study and the lack of confidence in the results justify for a reliability factor of 3.

Overall conclusion Acute Toxicity Invertabrates.

Two studies are available to evaluate the acute toxicity of cypermethrin to invertabrates. The Manson study which is considered reliable and the Stephenson study which suffers from many deficiencies and is considered of low reliability.

The 48h EC_{50}= 4.71µg/L.

5.4.2.2 Long-term toxicity to aquatic invertebrates

Table 515: Summary of long-term toxicity studies to aquatic invertebrates

Guideline/ Test method	Spp.	Exposure		Results (mg a.s./L) measured			Reference
		design	duration	LOEC	NOEC	EC_{50} (21-d, reprod)	
OECD 202, part II. Reduction in reproduction test. GLP = Yes Rel = 1	*Daphnia Magna*	Semi-static	21d	0.2µg/L	0.04µg/L	0.35µg/L	*Dickhaus, S.* 1990, Pharmatox, Report E.H./B. 2-7-44-90 (CYP/T143)
				LOEC	NOEC	EC_{10} (95% CL)	
OECD 211, Reproduction test. GLP = Yes Rel = 1	*Daphnia Magna*	Semi-static	21d	194.77µg/L	0.053µg/L	0.0879µg/L	*Simon M.* 2015, *Daphnia magna*, reproduction test (oecd 211); semi-static exposure - effect of cypermethrin 40/60 technical on the reproduction of *Daphnia magna*

From CAR (biocide)

Dickhaus, S. (1990) has studied the effects of cypermethrin cis:trans/40:60 on the reproduction and growth rate of *Daphnia magna* in a 21 day study according to the OECD guideline 202 part II.

A semi-static study design was used with test solution renewals performed every 48 hours. Live and dead offspring from the parental generation were counted and dead specimens removed either daily or at least 3 times per week (with an interval of 48-72 hours). Newborn young from the F1 generation were counted at least 3 times per week (with an interval of 48-72 hours) and a visual assessment of their condition recorded before the young were poured away. Only the parental animals were put into the renewal solution, animals from each F1 generation were poured away after counting and examination. Four different test concentrations of 0.008, 0.04, 0.2 and 1µg/L cypermethrin were tested along with a water control. The test item concentration were analysed on day 1, 7, 14 and 21. Refinding rates were means 94.9% (sd = 4.0%). Oxygen content of the test vessel was monitored on a daily basis and appears to be constant (0.8 mg/L). Results are express as reduction in reproduction. The NOEC and EC_{50} were calculated based on the nominal concentration according to the result showed in table 62. The EC_{50} was calculated by probit analysis with Gauss' Integral.

Table 526: Summary of individual findings after 21 days (*Dickhaus S.*, 1990)

Test concentration (µg/L cypermethrin)	Day first litters appeared	Total no. of newborn young (mean)	Reduction in reproduction (%)	No. of mortalities in P generation	Mortality rate in P generation (%)
0.000 (control)	7	109.8 ± 8.26	-	1	2.5
0.008	7	110.0 ± 10.27	-	2	5.0
0.040	7	101.9 ± 9.53	7.2	4	10.0
0.200	9	78.1 ± 10.40	28.9	14	35.0
1.000	11	41.4 ± 16.47	62.6	23	57.5

Conclusion:

Results indicated that cypermethrin cis:trans/40:60 has a chronic toxicity to *Daphnia magna*, with a NOEC = 0.04µg/L (reduction of reproduction) and an EC_{50}= 0.35µg/L (reproduction).

Simon M., (2015), determined the effects of cypermethrin technical (cis:trans, 40:60) on the immobilisation, reproduction, and growth of the *Cladoceran Daphnia magna* during a 21 day exposure in a semi-static test design according to OECD 211.

Method:

Nominal test concentrations of 4.0, 12.8, 40.0, 128 and 400 ng a.s./L (corresponding to time weighted average (TWA) of mean measured initial and mean measured aged concentration at test solution renewal were 3.11, 6.86, 19.08, 53.15 and 194.77 ng a.s./L) were prepared for the test with the addition of a negative control (without the test item) and a solvent control (acetone). Each treatment group consisted of 10 replicates with one daphnid each (individual exposure).

Control and solvent control were compared using Student-t test, Fisher`s Exact Binomial test, or Welch-t test depending on variance homogeneity. For each endpoint, the NOEC, LOEC, and, if possible, the EC_{10} and EC_{20} was determined. A LOEC was calculated by using ANOVA followed by Dunnett's or Williams' test or an appropriate non-parametric test. When the test results show a concentration-response relationship, the data are analysed by regression to determine the EC_{10} and EC_{20} including the 95 % confidence interval using Probit-analysis assuming log-normal distribution of the values. Due to mathematical reasons or inappropriate data EC_x calculation could not be applied for adult survival, growth and the number of offspring per surviving parent.

Deficiences:

Deviating from the study plan, the newborn daphnids per beaker were counted and removed daily only until day 12. After day 12 newborn daphnids were counted and removed three times weekly at each water renewal. According to the guideline, daily removal and counting of offspring is preferred but not mandatory. However, reproduction meets the validity criterion and a healthy growth was observed in both control groups. Therefore, this deviation is assumed to be without influence on the validity of the study and plausibility of the results. Deviating from the study plan, the concentration in the treatment with a nominal concentration of 4 ng/L was 8 ng/L for the first 9 days. This was due to a technical mistake at preparation of this application solution. However, the mean measured concentration was always below the mean measured concentration of the second treatment concentration. Therefore, a concentration series was still maintained. Additionally, no biological effects were observed in the three lowest treatments. Therefore, this deviation is assumed to be without influence on the validity of the study and plausibility of the results.

Deviating from the guideline, in aged medium pH surpassed 9 and reached 10 after 12 days. This was due to the relatively high pH of 8 of the dilution water (purified drinking water) and the feed algae loading increase with time, independent from test item concentration. This pH increase in aged medium was maintained stable by stopping the feed increase planned until test end at day 14. To ensure a valid reproduction rate a feed rate of 0.2 mg C/Daphnia/day equivalents had been intended for the last week of the study since the specimens grow. However, since the reproduction rate was above the validity criterion and a healthy growth was observed in both control groups, the deviation is assumed to be without influence on the validity of the study and the plausibility of the results.
Deviating from the guideline, total hardness was not measured at least once a week, in fresh and old media, in the control(s) and in the highest test substance concentration. In spite of the passage through a lime-stone column, hardness of the purified drinking water applied at the test facility for Daphnia tests is below the recommended (not required) range (140 – 250 mg/L as $CaCO_3$) for *Daphnia magna* indicated in the guideline. Historical data showed values of 70 – 120 mg/L as $CaCO_3$. However, according to the guideline lower hardness may be also appropriate for other Daphnia species. Historical results from reference tests at the test facility indicate that test conditions are also suitable for the *Daphnia magna* strain used at the test facility. Due to the consistent values in the past, values for total hardness are indicated for the last regular measurement before test start and the first regular measurement after test end. No measurement in the course of the study is applied. Due to the very low test item concentrations, it is assumed that total hardness is not affected by the test item. However, since the reproduction rate was above the validity criterion and a healthy growth was observed in both control groups, the deviation is assumed to be without influence on the validity of the study and the plausibility of the results.

Results:

Analytical
The mean measured test item concentrations of the freshly prepared test solution (initial concentrations) were between 85 % and 147 % of nominal concentrations. During the time interval until renewal of the test solution, test item concentrations decreased considerably to 18 - 38 % of nominal. Analyses of fresh and aged exposure media revealed a significant decrease of the test item within the exposure intervals. Therefore, time weighted average concentrations were calculated for the evaluation of the biological parameters and the endpoints (Table 65).

Table 537: Concentrations of the Cypermethrin technical (cis:trans, 40:60) (*Simon M.*, 2015)

Nominal concentration [ng a.s./L]	Mean measured initial concentration [ng a.s./L]	% of nominal	Mean measured aged concentration [ng a.s./L]	% of nominal	Time weighted mean conc. [ng a.s./L]	% of nominal
4*	5.86	146.6	1.52	38.0	3.11	77.8
12.8	12.11	94.6	3.68	28.8	6.86	53.6
40	41.91	104.8	7.62	19.1	19.08	47.7
128	108.61	84.9	22.86	17.9	53.15	41.5
400	338.36	84.9	103.39	25.8	194.77	48.7

* The concentration in the treatment with a nominal concentration of 4 ng a.s./L was 8 ng a.s./L for the first 9 days due to a technical mistake at preparation of this application solution.

Biological

No effect on parental survival was observed. The EC_{10} and EC_{20} were found to be > 194.77 ng a.s./L based on time weighted average concentrations (TWA). The NOEC mortality was found to be ≥ 194.77 ng a.s./L TWA. No other clinical signs were observed in any replicate at any concentration tested. Adult body length exhibited no significant differences between treatments (NOEC growth ≥ 194.77 ng a.s./L TWA). Neither any physical nor pathological symptoms were obtained. There were no statistically significant differences between control and solvent control. Data are presented in Table 66.

Time to first brood was between 9.9 and 11.7 days up to the fourth concentration tested. No statistically significant effects were observed up to this concentration level (NOEC = 53.15 ng a.s./L TWA). With a time to first brood of 14.3 days, a significant delay of reproduction started at the highest concentration tested (LOEC = 194.77 ng a.s/L TWA). Due to mathematical reasons, no EC_x calculation can be applied to the endpoint time to first brood. Time to first brood was not inhibited by the solvent.

The cumulative number of offspring per female ranged from 67 to 80 up to the fourth concentration tested. No statistically significant effects were observed up to this concentration level (NOEC = 53.15 ng a.s./L TWA). With a mean number of offspring of 39, a significant decrease started at the highest concentration tested (LOEC = 194.77 ng a.s./L TWA). Offspring was approximately 10 % lower in the first four treatment groups when compared to the solvent control (see Table64). However, this decrease was not statistically significant and did not follow a dose-response-relationship. In contrast, the decrease by 48 % at the highest treatment level of 194.77 ng a.s./L must be explained by test item effect. However, due to the insufficient data set, it was not possible to estimate EC_x values. Anyway, an EC_{10} of ≥ 53.15 ng a.s./L TWA can be assumed. There were no statistically significant differences between control an solvent control.

The intrinsic rate of population increase ranged from 0.276 and 0.300 up to the fourth concentration tested (Table 66). No statistically significant effects were observed up to this concentration level (NOEC = 53.15 ng a.s./L TWA). With an intrinsic rate of 0.216, a significant decrease started at the highest concentration tested (LOEC = 194.77 ng a.s./L TWA). A concentration-response relationship started with the fourth treatment. The EC_{10} and EC_{20} were calculated to be 87.99 ng a.s./L TWA (95% CL: 83.96 - 91.80) and 148.38 ng a.s./L TWA (95% CL: 145.0 - 151.7), respectively.
Survival, growth reproduction and intrinsic rate of population data of *Daphnia magna* after 21 days and their corresponding endpoints.

Table 548: Result of immobilisation, reproduction, and growth of *D. magna* (*Simon*, 2015)

Concentration [ng a.s./L TWA]	Parental survival [%]	Growth (length) [Mean ± SD] (mm)	Time to first brood [Mean ± SD] (days)	Cumulative offspring per female [Mean ± SD] (Ind.)	Intrinsic rate of population increase [Mean ± SD] (Ind./day)
Control	100	4.77 ± 0.48	11.7 ± 1.0	79.9 ± 17.5	0.276 ± 0.018
Solvent control	90	4.64 ± 0.29	10.8 ± 2.0	76.2 ± 14.4	0.295 ± 0.025
3.11	100	4.65 ± 0.18	9.9 ± 0.5	69.8 ± 10.2	0.297 ± 0.012
6.86	100	4.73 ± 0.20	10.0 ± 1.1	68.9 ± 8.5	0.300 ±0.023
19.08	100	4.82 ± 0.28	10.0 ± 0.9	66.7 ± 12.9	0.295 ± 0.023
53.15	100	4.84 ± 0.19	10.9 ± 1.3	69.2 ± 10.6	0.282 ± 0.022
194.77	100	4.43 ± 0.30	14.3 ± 1.8 *	39.3 ± 18.1 *	0.216 ± 0.033 *
Endpoints [ng a.s./L TWA]					
EC_{10} (95% CL)	n.d.	n.d	n.d. [1)]	n.d.	87.99 (83.96 – 91.80)
EC_{20}	n.d.	n.d.	n.d. [1)]	n.d.	148.38 (145.0 – 151.7)
NOEC	≥ 194.77	≥ 194.77	53.15	53.15	53.15

Mean ± standard deviation (SD)
* significant deviation from solvent control
n.d. = not determined due to mathematical reasons and / or inappropriate data
[1)] Time to first brood does not allow to calculate ECx-values because it is not possible to define the effect size x (maximum possible increase in age not known)

Conclusion

Cypermethrin technical (cis:trans, 40:60) showed chronic adverse effects on reproduction of *Daphnia magna* under the chosen test conditions. Adult survival and growth (body length) were not affected up to the highest concentration tested (NOEC survival ≥ 1.9477E^{-4} mg a.s./L TWA and NOEC growth ≥ 1.9477E^{-4} mg a.s./L TWA). The NOEC for reproduction (number of offspring per surviving parent and time to first brood) and intrinsic rate of population increase was determined to be 5.315E^{-5} mg a.s./L TWA. The EC_{10} and EC_{20} for intrinsic rate of population increase were calculated to be 8.799E^{-5} mg a.s./L TWA (95% CL: 83.96 - 91.80) and 1.4838 E^{-4} g a.s./L TWA (95% CL: 145.0 - 151.7), respectively. EC_x calculation could not be applied for adult survival, growth and the number of offspring per surviving parent.

Overal conclusion on chronic toxicity to invertabrates.

Two studies is available, dickhaus, 1990 and Simon, 2005. Both studies provide reliable results wich are in the same range.

A NOEC of 4E^{-5} mg/L (0,00004 mg/L) can be set based on basis of the dickhaus,1990 study.

5.4.3 Algae and aquatic plants

Table 559: Algae and aquatic plants studies with cypermethrin

Guideline / Test method	Spp.	Exposure duration	Results (µg a.s./L) measured			Reference
			EbC50	ErC50	NOEbC	
OECD 201 GLP = Yes Rel= 1	*Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*)	96 hours	>33.0µg/L	>33.0µg/L	= or >33.0µg/L	*Manson*, 2005c, Covance report 1669/020
OECD 201 GLP= no Rel=3	*Selenastrum capricornutum*	96h	/	>100µg/L	/	*Stephenson*, 1981, WL 85871 and Cypermethrin: A Comparison of Their Acute Toxicity to *Salmo gairdneri*, *Daphnia magna* and *Selenastrum capricornutum*.

From CAR (Biocides)

In 2006, *Manson* studied the inhibition effet of cypermethrin cis:trans/40:60 on the growth of the alga *Pseudokirchneriella subcaptitata,* according to the OECD guideline 201 in a limited test. The tested concentration was above the solubility value of the test item (4µg/L).

The test item concentrations were monitored at 0 and 96h for the control, the solvent control (acetone) and from inoculated and non-inoculated vessels. Based on the geometric mean measured concentration of cypermethrin cis:trans 40:60, the 24, 48, 72 and 96-hour ErC_{50} and the EbC_{50} toxicity values could not be calculated as no significant inhibition of algal cell growth occurred during the definitive test in either of the test parameters (the area under the growth curves (A) or the average specific growth rates (µ)) relative to the control.

The 24, 48, 72 and 96-hour ErC_{50} and EbC_{50} toxicity values are considered to be > 33.0 µg/L, the geometric mean measured concentration. The corresponding NOEC values were observed to be ≥ 33.0 µg/L.

Conclusion:

Cypermethrin cis:trans/40:60 doesn't show toxicity to algae at concentration **above** the water solubility value.

From DAR (PPP)

Stephenson, (1981): WL 85871 and cypermethrin: A Comparison of Their Acute Toxicity to *Salmo gairdneri, Daphnia magna* and *Selenastrum capricornutum*.

Guidelines :

OECD guideline 201

GLP : No

Material and Methods :

Test substance :

cypermethrin, chemical purity: 98.0-98.5

alpha-cypermethrin, chemical purity: 93.4-95.7

Test species : *Selenastrum capricornutum*

Number of organisms: 5.10^3 cells/ml, 2 replicates per test concentration

Type of test: 96 hours static test

Applied and measured concentrations :

nominal : acetone control,1, 10 and 100 µg/l.

The analysis of the test solutions showed that there was a mean decline of 35% and 39% in the concentration of cypermethrin and alpha-cypermethrin between start and end of the test.

Initial concentrations in test media were 82-73% and 90-120% of the nominal values (100 µg/L) respectively for cypermethrin and alpha-cypermethrin, After 96 hours the water recoveries were 49-51% and 57-74% respecitively for cypermethrin and alpha-cypermethrin (sum of the a.s. and metabolite WL86711).

Test conditions :

temperature : 24 ± 1°C

pH : -

oxygen content : -

total hardness : -

Photoperiod : constant illumination, intensity is not mentioned

Analytical methods : gas chromatography

Findings and conclusions :

The quality of the study is limited.

ErC_{50} (48-96h, cypermethrin) > 100 µg a.s./l

This endpoint is less critical than the daphnia and fish endpoints, and is also covered by the mesocosm endpoint. The algae endpoint is therefore not expected to determine the labelling nor the M-factor for cypermethrin.

Comment RMS: This study is the same study as for the Daphnia acute endpoint. The study suffers of the same deficiencies: GLP and quality insurance missing wich ; identity not fully known; low recovery rates; lack of stability (please refers to extended discussion under 5.4.2, page 107). Therefore this lack of confidence in the result allows for a reliability of 3 for the invertebrates endpoints. As a matter of consistency, the outcome of the study for algea should also be attributed a reliability factor of 3. But RMS aknowledge that in this case, this is less critical.

Overal conclusion for Plant and Algea

Two studies are available, *Manson*, (2006) and *Stephenson*, (1981).

The first study is a state of the heart study wich provide reliable results. From this study, a NOEbC= 0.33µg/L can be extracted.

5.4.4 Other aquatic organisms (including sediment)

5.4.4.1 Microbial activity

Table 70: Inhibition of microbial activity study (aquatic)

Guideline/ Test method	Spp.	Exposure duration	Results (mg a.s./L) measured		Reference
			EC50	NOEC	
OECD 209 GLP = Yes Rel = 1	Activated sludge	3hr	163	n.d.	*Bealing D.*, 2002, Covance study no 40/46-D2149

Bealing D., (2002) determined the inhibition of respiration of activated sludge by cypermethrin cis:trans/40:60. In this study, due to the low water solubility and immobility at room temperature a formulation containing 50% cypermethrin cis:trans/40:60 in emulsifier surfactant was used.

Samples of activated sludge (from a predominantly domestic sewage plant source) were exposed to various nominal concentrations of cypermethrin (purity 96.5%) as a 50% a.i. formulation, ranging from 1.0 to 1000 mg a.i./L in a range-finding experiment and from 50 to 500 mg a.i./L in a definitive test. Their respiration rates were measured after 3 hours contact time. The formulation blanks showed no significant inhibition when dosed at 482 mg Ethylan C12 AH/L, corresponding to the top formulation dose, confirming that inhibition was caused by cypermethrin alone.

No significant respiration inhibition occurred in formulation blanks that contained the emulsifier at the highest formulation concentration tested. The effective concentration of cypermethrin that caused a 50% reduction in respiration rate relative to untreated controls (EC50), was 163 mg /L (95% confidence limits: 118 to 230 mg/L).

Conclusion:

Cypermethrin cis:trans/40:60 shows 50 % inhibition of the microbial respiration at effective concentration of 163 mg/L.

EC_{50}= 163 mg/L which is far above the water solubility (4µg/L).

5.4.4.2 Mesocosm study

Table 71: Mesocosm study with cypermethrin

Guideline/ Test method	Species.	Test material conc. (µg/L)	Radio-labelled	Exposure		Result Min NOAEC	Remarks	Reference
				design	duration			
No: Study conducted to support the agricultural use of cypermethrin, taking into account the recommendations of workshops on higher tier risk assessments for aquatic organisms (e.g. HARAP, CLASSIC). GLP = Yes Rel = 1	Multiple	0, 0.0016, 0.005, 0.016, 0.05, 0.2, 1	No	2 appl. separated by 14d	6 months	0.05µg/L		*Schnoder, F.* (2003), Covance Laboratories Ltd, study no. 0040/045 (CYP/T331),

The effect of the use of Cyperkill 10, EC (containing 100 g/L cypermethrin technical) on naturalized ecosystems was investigated in an outdoor pond study by Schnoder, F. (2003).

The enclosures were treated twice with 0, 0.0016, 0.005, 0.016, 0.05, 0.2 and 1.0 µg a.s./L. with a minimum 14d interval (14 may 2002, day 0 and 28 may 2002). The test item was applied below the surface, directly into the water column. There were 6 replicates for the control, 3 replicates for the treatment groups 0.0016 to 0.05 and 2 replicates for treatment groups 0.2 and 1 µg a.s./L.

Each pond contained 5 cm clay layer with a 10-15 cm overlying layer natural sediment. The water depth of the pond was approximately 1.1 m.

Biological samples were collected both before and after treatment with last sampling on day 111. Abundance data were analyzed for 5 main categories of test organisms: zooplankton, emergent insects macrozoobenthos, phytoplankton and periphyton and for 2 additional data types: Chaoborus (combined sampling techniques), and blue-green algae. Principal Response Curves, NOEC and EC_{50} were produced for each data category.

Water samples were analysed for cypermethrin using gas chromatography with electron capture detection (GC/ECD) and gas chromatography with mass spectroscopy (GC/MS), for all enclosures following each application. In addition, water and sediment samples from selected enclosures at 1.0, 0.05 and 0.005 µg cypermethrin/L were analysed to determine the dissipation of cypermethrin. All enclosures were monitored for physical and chemical parameters of the water at appropriate weekly or bi-weekly intervals.

In all except two enclosures, measured cypermethrin concentrations in the test enclosure water samples where the nominal concentrations were above the limit of quantification (0.01µg/L), taken 2 hours after each treatment, ranged from 103% to 140% of nominal. The two enclosures whith concentrations outside of this range were the test enclosure dosed at 0.016 µg/L where the measured concentration was 150% of nominal after the first application and the test enclosure dosed at 1.0 µg/L where the measured concentration was 74% of nominal after the second application. These results indicate that treated enclosures were dosed correctly and the target exposure concentrations were achieved. No cypermethrin was detected in control enclosures.

Measured concentrations of cypermethrin cis:trans/40:60 in the water samples from enclosures monitored at 0.05 µg/L and 1.0 µg/L declined rapidly over time. The estimated water column DT_{50} values were 22.3 hours and 20.9 hours for nominal concentrations 0.05 µg/L and 1.0 µg/L respectively.

Cypermethrin concentrations in sediment were analysed in selected enclosures at 0.05 µg/L and 1.0 µg/L. Measured concentrations (as dry weight equivalents) for total cypermethrin in sediment samples at 0.05 µg/L were below the limit of detection (LOD) of 0.41 µg/Kg during the whole course of the study. Cypermethrin concentrations in sediment at 1.0 µg/L ranged from 1.88 µg/Kg on day 4 to a peak of 6.77 µg/Kg on day 16 (2 days after the second application). While there was some variability in the measured concentrations (samples were below the LOQ of 0.79 µg/Kg on days 2, 18 and 42), which may reflect heterogeneity in the sediment sampling, measured sediment concentrations declined to 1.42 µg/Kg (2 x the LOQ) by day 84 (last sediment sampling date).

Zooplankton :

Table 562: Summary of NOEC values < 1.0 µg a.s./L, including an overall NOEC/NOAEC, for Zooplankton (*Schnoder F.* ,2003)

Day of study	Total Zooplankton	Order Cladocera	Family Daphniidae	Daphnia longispina	Genus Chydorus	Sub-class Copepoda	Family Cyclopidae	nauplii	Class Rotatoria	Genus Polyarthra	Genus Synchaeta	Family Synchaetida	Species Keratella quadrata	Genus Chaoborus
1						0.005		0.005						
3	0.016	0.016	0.016	0.016		0.005	0.016	0.005						<0.0016
7	0.2	0.016	0.016	0.016		0.005	0.005	0.005						0.005
14	0.2					0.05	0.016	0.05	0.2					0.005
15						0.05		0.05						N/A
17	0.2					0.05		0.05						<0.0016
21	0.2					0.05		0.05	0.2				0.2	<0.0016
28						0.05	0.05	0.2						<0.0016
35	0.05					0.05	0.2	0.05	(<0.0016)		0.0016	0.2	0.0016	<0.0016
42		0.05				0.05		0.05			<0.0016	0.2	0.016	<0.0016
49						0.2	0.2	0.2						0.2
56						0.2	0.2							0.2
63		(0.005)			0.016	0.2	0.2							0.2
70							0.2						0.2	
77													<0.0016	
84				See bioassay										
98							0.2							
Overall NOEC	0.016	0.016	0.016	N/A	0.05	0.005	0.005	0.005	0.2	0.2	<0.0016	0.2	0.016*	<0.0016
NOAEC	1.0	**0.05**	**0.05**		**0.05**	1.0	0.2	1.0			**0.05**		**0.05**	0.2

Zooplancton community has been shown to be both abundant and diverse during the study and no species were eliminated in any enclosure concentration during the study following application of cypermethrin. The lowest overall NOEC community determined by Principal Response Curve analysis was 0.0 5 µg a.s./L. The Principal Response Curve (PRC) analysis indicated a recovery from day 56 onwards at 0.2 µg a.s./L. At the 1.0 µg a.s./L the PRC analysis indicated no recovery by the end of the study. A general change in the population structure of *Daphnia longispina* was not considered treatment-related but rather due to seasonal and successional reasons. Results of the bioassay conducted at day 86 showed mortality in the control and in the treatments were in the range 10-17%. No dose response was observed indicating that on day 86 *D. longispina* would survive in all test enclosures. At nominal concentrations of 0.0016µg/L and 0.005µg/L, the PRC analysis indicated no difference from the controls during the study. Therefore no dose related effect is identified. Data on individual were considered unreliable due to low abundance in both control and treated enclosure.

Emergent insects :

Table 573: Summary of all NOEC values < 1.0 µg a.s./L (NOEC = 1 µg/L in the empty boxes) for Emergent insects (*Schnoder F.*, 2003)

Day of study	Class Insecta	Order Dipterea (*)	Family Chironomidae (*)	Sub-family Chironomimae (*)	Genus Chironomus (*)	Sub-family Orthrodiinae (*)	Genus cricotopus (Isocladius) (*)	Genus Corynoneura (*)	Genus Chaoborus (*)	Order Coleoptera	Family Baetidae
3+7	0.2										
14											
17+21											
28	< 0.0016										
35											
42	0.2										
49	0.2										
56											
63					0.2						< 0.0016
70											
77											
84											
91										(0.2)	
98											
105											
Overall NOEC	1.0	0.2	0.2	1.0	0.2	1.0	1.0	1.0	<0.0016	1.0	0.016
NOAEC	1.0								**0.05 ~~(0.2)~~**	1.0	**0.05 ~~(0.2)~~**

Statistical analysis resulted in NOEC of 1 µg/L for almost all taxonomic groupings on all sampling occasions. These NOEC were considered unreliable due to low abundances in both control and treatment enclosures. The Principal Response Curve analysis indicated no significant treatment-related deviation from the control up to day 35. Although the deviation from the control increased from day 42 onwards a dose response was not apparent. The lowest overall NOEC community for emergent insects was determined to be 0.005µg/L but should be treated with some caution due to low abundances in both control and treated enclosures. The NOAEC was estimated to be ≥1.0 µg/L. Individual NOEC were considered unreliable due to low abundance in both control and treated enclosure.

Phytoplankton :

Table 74: Summary table of all NOEC values < 1.0 µg a.s./L for Phytoplankton

Day of study	Total phytoplankton	Phylum Chrysophyta	Class Bacillariophyceae	Family Nitzschiaceae	Class Chrysophyceae	Family Synuraceae	Family Chromulinaceae	Class Chlorpphyceae	Family Chlorelallaceae	Genus Monoraphidium
3			0.2	0.2						
13	<0.0016		0.2				0.2		0.2	
17	<0.0016									
28	0.016	0.0016	0.005	0.2		0.005				
56	0.005	0.005	0.016	0.2						
70	0.005									
Overall NOEC	<0.0016	0.0016	0.005	0.2	1.0	0.005	1.0	1.0	0.016	1.0
NOAEC	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

No apparent treatment related effects were observed by the delayed fluorescence technique. Transient treatment related effects were observed by microscope counting. The lowest overall NOEC community was 0.005 µg a.s./L.

The Principal Response Curve analysis indicated a deviation from the control at 0.016, 0.05, 0.2 and 1.0 µg a.s./L on day 28. At day 56 and 70, the deviation appears not to be dose-related (NOAEC = 1 µg a.s./L).

Macrozoobenthos. The macrozoobenthos community was abundant and diverse throughout the study. Abundance for the whole group of macrozoobenthos was not affected at the three lower levels, but a decrease in abundance at the three upper levels was observed after the first application. Population recovery at 0.05 µg/L and 0.2 µg/L occurred by day 14, with recovery at 1.0 µg/L occurring by day 70. An overall NOEC < 0.05 µg/L was determined for several species. Those species with an overall NOEC lower than 0.05 µg/L were *Chironomidae* and the *Baetidae* with an overall

NOEC of 0.005 µg/L and *Planorbidae* with an overall NOEC of 0.016 µg/L. *Diptera* had an overall NOEC of 0.05 µg/L. A NOEAEC of 0.05 µg/L was justified for the Macrozoobenthos community because effects were only transient and, therefore, considered of minor ecological relevance. No species was eliminated from any enclosure at any treatment level.

Table 585: Summary table of all NOEC values < 1.0 µg a.s./L (NOEC = 1 µg/L in the empty boxes) for Macrozoobenthos (Schnoder F., 2003)

Day of study	4	8	13	18	22	29	36	43	50	57	64	71	78	85	Overall NOEC	NOAEC
Family Planorbidae (Gastropodaé)	0.016	0.0016		0.2			0.2								0.016	1.0
Family Baetidae (Ephemeroptera)		<0.0016					0.0016	0.005	0.005	0.005	0.05	0.2	0.2	0.2	0.005	**0.05**
Family Chironomidae (Diptera)		**0.005**		**0.2**	**0.2**	**(<0.0016)**	**0.05**	**0.2**	**0.2**	**0.2**					**0.005**	**0.2**

Periphyton. An overall NOEC < 0.05µg/L was determined only for one taxon. The diatoms showed an indirect treatment-related increase at the five upper treatment levels at day 14 only. The blue-green algae (*Cyanophyceae*) were affected at the five highest treatment levels (0.005µg/L to 1.0µg/L) at day 14 and at all treatment levels at day 18. Full recovery of populations was observed by day 29. However it is notable that these indirect effects were not observed for the blue-green algae counted by microscope. The class *Chrysophyceae* and the family *Chroococcaceae* were affected at 0.016µg/L to 1.0 µg/L and at 0.2µg/L to 1.0µg/L respectively, at day 4, however both taxa showed a complete and rapid recovery by day 14. Based on the full and rapid recovery the proposed NOEAEC was 0.05µg/L.

Table 596: Summary table of all NOEC values < 1.0 µg a.s./L for Periphyton (Schnoder F., 2003)

Day of study	Total Periphyton	Class Bacillariophycaed	Family Nitzschiaceae	Class Chlorophycea	Genus Chlorella	Family Chlamydomonaceae	Family Dictyosphaericaceae	Family Tetrasporaceae	Family Coleochaetaceae	Class Chrysophyceae	Class Euglenophyceae	Class Cyanophyceae	Class Chroococcaceae	Class Oscillatoriaceae
4										0.005			0.05	
14														
18														
29														
Overall NOEC	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.005	1.0	1.0	0.05	1.0
NOAEC	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

Macrophytes :

No apparent treatment-related effects were observed. The NOEC and NOAEC were 1.0 µg a.s./L.

Conclusion:

In this highest tier study, no dose response related effect is identifiable after twice application of cypermethrin in an artificial pond for zooplankton and for emergent insect. An NOAEC of 0.05µg/L was determinated for the macrozoobenthos community. An overall NOAEC of 1 µg/L was calculated for the phytoplankton and of 0.05µg/L for the periphyton. The macrophytes were characterized by an NOAEC of 1.0 µg/L.

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Degradation :

The result of the test on the biodegradations of cypermethrin shows that cypermethrin is considered not rapidly degradable for purpose of classification and labeling.

Bioaccumulation :

Cypermethrin has a Log K_{ow} of 5.6. The experimentally derived BCF is 373(+/- 45) L/Kg which is below the trigger of 500.

Aquatic tocity :

The lowest EC_{50} values for cypermethrin are between > 0.001 < 0.01 mg/L for fish (0.00283 mg/L); > 0.001 < 0.01 for crustacean (0.0047 mg/L) and > 0.01 < 0.1 mg/L for algae (>0.033 mg/L). Chronic NOEC values are between >0.0001<0.001 for fish (0.00025 mg/L), > 0.00001 < 0.0001 mg/L for crustacean (0.00004 mg/L) and > 0.01 mg/L for algae (≥0.033mg/L). A mesocosm study produces values NOAEC > 0.00001< 0.0001mg/L for macrozoobenthos community and periphyton.

Based on the lowest LC_{50} (fish), cypermethrin should be classified as **Aquatic Acute Category 1 and an M factor of 100 is proposed.**

NOEC values for cypermethrin are available for all trophic levels. The lowest acceptable NOEC is -0.00004 mg/L (obtained for invertebrates). Cypermethrin fulfills criteria for classification as **Aquatic Chronic Category 1.**

The lowest NOEC is between 0.00001 mg/l and 0.0001 mg/l and Cypermethrin is considered not rapidly degradable, therefore an M factor of 1000 for chronic toxicity is proposed.

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

Cypermethrin fulfils the criteria for classification as Aquatic Acute Cat. 1 with an M-factor of 100.

Cypermethrin fulfils the criteria for classification as Aquatic Chronic Cat.1 with an M-factor of 1000.

6 OTHER INFORMATION

6.1 Hazardous to the ozone layer

See section 5.1.1. Stability, Photochemical degradation in air.

7 REFERENCES

Table 607: Références

Author(s)	Year	Title Source (where different from company) Report no. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
Aldana L González de Mejía E., Craigmill A., Tsutsumi V., Armendariz-Borunda J., Panduro A., Rincón A.R.	1998	Cypermethrin increases apo A-1 and apo B mRNA but not hyperlipidemia in rats. Toxicoligy Letters 95: 31-39.		
Barnes S.	2005	Cypermethrin cis:trans/40:60 Evaluation of ultimate anaerobic biodegradability by measurement of biogas production Huntigdon Life Sciences Ltd., report no. HZL 010/053287 GLP, unpublished	Yes (Exist./First)	CAG
Bates, M	2002a	Cypermethrin cis:trans 40:60 (purified active substance) : Evaluation of the physico-chemical properties Covance Laboratories Ltd, report no. 40/30-D2149 (CYP/C65) Chimac-Agriphar S.A., document no. KIIA, 2.0/01 GLP, unpublished	Yes (Exist./First)	CAG
Bates, M	2002b	Cypermethrin cis:trans 40:60 (technical active substance) : Evaluatio of the physico-chemical properties Covance Laboratories Ltd, report no. 40/33-D2149 (CYP/C63) Chimac-Agriphar S.A., document no. KIIA, 2.0/02 GLP, unpublished	Yes (Exist./First)	CAG

Author(s)	Year	Title Source (where different from company) Report no. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
Bealing, D.	2002	Cypermethrin – Determination of inhibition of respiration of activated sludge Covance Laboratories Ltd., report no. 40/46 (CYP/T323) Chimac-Agriphar S.A., document no. KII A, 8.7/01 GLP, unpublished	Yes (Exist./First)	CAG
[redacted]	1985	Acute Aerosol Inhalation Toxicity in the Rat of CGA 55186 Tech. (cypermethrin). Ciba-Geigy Ltd, report No.:840047 (CYP/T82g) Chimac-Agriphar S.A., document no. KII A, 5.2/01 Non-GLP, unpublished	Yes (Exist./First)	CAG
Brice A, Cooke C	2006a	[^{14}C]-Cypermethrin cis:trans 40:60: Degradation and retention in water-sediment systems. Covance Laboratories Ltd, Report No. 1669/014-D2149 GLP, unpublished	Yes (Exist./First)	CAG
Brice, A., Cooke, C.	2006b	[^{14}C]-Cypermethrin cis:trans 40:60: Adsorption/Desorption in soil Covance Laboratories Ltd., report no. 1669/015-D2149 GLP, unpublished.	Yes (Exist./First)	CAG
Brice, A. Cooke, C.	2006c	[^{14}C]-Cypermethrin cis:trans 40:60: Aerobic soil degradation and metabolism Covance Laboratories Ltd., report no. 1669/012-D2149 GLP, unpublished	Yes (Exist./First)	CAG
Brice, A. Cooke, C.	2006d	[^{14}C]-Cypermethrin cis:trans 40:60: Anaerobic soil degradation and metabolism. Covance Laboratories Ltd., report no. 1669/013-D2149 GLP, unpublished	Yes (Exist./First)	CAG

Author(s)	Year	Title Source (where different from company) Report no. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
[redacted]	1977	A 13 week feeding study of WL 43467 (cypermethrin) in dogs. Shell UK Ltd., report no. TLGR.77.127 (CYP/T9). Chimac-Agriphar S.A. , doc. No. KII A, 5.3.2.2/01.		
Burwood, C.	2005	Cypermethrin cis:trans/40:60: Assessment of Inherent Biodegradability by measurement of CO_2 evolution Covance Laboratories Ltd., report no. 1699/017-D2149 GLP, unpublished	Yes (Exist./First)	CAG
Cantalamessa F.	1993	Acute toxicity of two pyrethroids, permethrin, and cypermethrin in neonatal and adult rats. Short communication. Arch Toxicol. 67: 510-513.		
[redacted]	1976	Toxicity studies on the insecticide WL 43467: Summary of results of preliminary experiments. Shell Toxicology Laboratories (Tunstall). Study reference no. TLGR.0104.76 (CYP/T2).		
De Ryckel, B.	2005	Physical and chemical properties and storage stability tests for Cypermethrin 100 EW. Agricultural Research Centre (CRA-W), Gembloux, Belgium, report ref. Chimac-Agriphar/FO20896/Ch.3174/2004/178. Chimac-Agriphar company ref. KIII A, 2.1/01. GLP, unpublished	Yes (New/First)	CAG

Author(s)	Year	Title Source (where different from company) Report no. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
Dickhaus, S.	1990	21-Days reproduction test with compound cypermethrin technical in Daphnia magna Pharmatox Beratung und Forschung GmbH, report no. E.H./B.2-7-44-90 (CYP/T143) Chimac-Agriphar S.A., document no. KII A, 8.2.5/01 GLP, unpublished	Yes (Exist./First)	CAG
El-Toukhy M.A., Girgis R.S.	1993	*In vivo* and *in vitro* studies on the effect of larvin and cypermethrin on adenosine triphosphatase activity of male rats. J. Environ. Sci. Health B 28: 599-619.		
Giray B., Gürbay A., Hincal F.	2001	Cypermethrin-induced oxidative stress in rat brain and liver is prevented by Vitamin E or allopurinol. Toxicology Letters 118: 139-146.		
Greenwood, J., Maudsley, L.	2003	Cypermethrin cis:trans/40:60 (purified active substance): Quantum yield analysis Covance Laboratories Ltd, study number 0040/034 (CYP/M70) Chimac-Agriphar S.A., document no. KII A, 2.9.3/01 GLP, unpublished	Yes (Exist./First)	CAG
[redacted]	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) Chimac-Agriphar S.A., document no. KII A, 5..3.2.1/01 Not-GLP, unpublished	Yes (Exist./First)	CAG
[redacted]	1981	Subacute dermal toxicity study in rabbits with technical cypermethrin. Shell international Chemical Company. CTL/P/588. In 91/414 DAR for Cypermethrin, Annex B, prepared by the BE CA.		

Author(s)	Year	Title Source (where different from company) Report no. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
Klein, W.	1990	Biodegradation – The modified sturm test Fraunhofer Institute für Umweltchemie und Ökotoxicologie, report no. FEI-001/3-11 (CYP/M50) Chimac-Agriphar S.A., document no. KII A, 7.2.1.3.1/01 GLP, unpublished	Yes (Exist./First)	CAG
[redacted].	2005	Cypermethrin cis:trans/40:60 Fathead Minnow, early Life Stage test Charles River Laboratories, report no. 805972 GLP, unpublished	Yes (Exist./First)	CAG
[redacted]	1984a	Acute Oral LD50 in the Rat of CGA 55186 Tech. (cypermethrin) – (administration in oily medium). Ciba-Geigy Ltd, report No.:840042 (CYP/T82b). Chimac-Agriphar S.A., document no. KII A, 5.2/01 Non-GLP, unpublished.	Yes (Exist./First)	CAG
[redacted]	2005a	Cypermethrin cis:trans/40:60: Acute toxicity to *Oncorhynchus mykiss*; Covance Laboratories Ltd; study no. 1669/018 GLP, unpublished	Yes (Exist./First)	CAG
Manson, P.	2005b	Cypermethrin cis:trans 40:60: Acute toxicity to Daphnia magna. Covance Laboratories Ltd., Report no. 1669/019-D2149 GLP, unpublished	Yes (Exist./First)	CAG
Manson, P.	2005c	Cypermethrin cis:trans/40:60: Inhibition of growth to the alga Pseudokirchneriella subcapitata Covance Laboratories Ltd, study no. 1669/020 GLP, unpublished	Yes (Exist./First)	CAG

Author(s)	Year	Title Source (where different from company) Report no. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
[redacted]	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) Chimac-Agriphar S.A., document no. KII A, 5.5.1/01 Not GLP, unpublished	Yes (Exist./First)	CAG
[redacted].	2005	Acute inhalation toxicity study with cypermethrin technical in wistar rats; Laboratory report / project number (Doc. No.): 4244/05 (523-001) GLP, unpublished	Yes	CAG
[redacted]	2006	[^{14}C]-Cypermethrin-cis:trans 40:60:- Absorption, Distribution and Excretion in the Rat Covance Laboratories Limited, report no. 1669/029 GLP, unpublished	Yes (Exist./First)	CAG
[redacted]	2005	Ravi, G.S. (2005); Acute oral toxicity study (acute toxic class method) with cypermethrin in Wistar rats; Rallis Research Centre, India, report no. 4242/05, 15 July 2005 (unpublished).	Yes (Exist/First)	CAG
Schneider , E.	1997	Hydrolysis in water at 3 pH values Dr Krebs Analytik GmbH, report no. PR97/003 (CYP/C52) Chimac-Agriphar S.A., document no. KII A, 2.9.1/01 GLP, unpublished.	Yes (Exist./First)	CAG
Schnoder, F.,	2003	*Evaluation of direct and indirect effects of Cyperkill 10 on aquatic organisms on outdoor enclosures (multi-site study) Covance Laboratories Ltd, study no. 0040/045 (CYP/T331*	yes	CAG
Simon	2015	Simon M. 2015, Daphnia magna, reproduction test (oecd 211); semi-static exposure - effect of cypermethrin 40/60 technical on the reproduction of Daphnia magna. GLP, unpublished	Yes (Exist./First)	AG

Author(s)	Year	Title Source (where different from company) Report no. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
_____	1981	WL85871 and cypermethrin: A comparison of Their Acute Toxicity to *Salmo gairdneri, Daphnia magna* and *Selenastrum capricornutum*	N	BASF
Swales, S.	2003a	^{14}C-Cypermethrin : Photodegradation in sterile, aqueous solution Covance Laboratories Ltd., Report N° 40/35 (CYP/M70) Chimac-Agriphar S.A., document no. KII A, 2.9.2/01 GLP, unpublished	Yes (Exist./First)	CAG
Swales, S.	2003b	(^{14}C)-cypermethrin: Photodegradation on a soil surface Covance Laboratories Ltd., Report N° 40/44-D2149 (CYP/M71) Chimac-Agriphar S.A., document no. KII A, 7.1.1.1.2/01 GLP, unpublished	Yes (Exist./First)	CAG
Sydney, P	2005a	Cypermethrin (pure) physicochemical properties Huntingdon Life Sciences Ltd, report no. CAV001/052563 GLP, unpublished	Yes (Exist./First)	CAG
[redacted].	1990	Draft report on flow-through test in rainbow trout to determine the bioaccumulation potential of cypermethrin Toxicological Research Centre, Report no. 90-016 (CYP/T133) Chimac-Agriphar S.A., document no. KII A, 8.2.3/01 *Not GLP, unpublished*	Yes	CAG
[redacted].	2012	Fish Early Life Stage Test (*Pimephales promelas*) with Cypermethrin; Covence Laboratories Study number 8252888	Yes	CAG

Author(s)	Year	Title Source (where different from company) Report no. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
[redacted]	1983	WL85871 and cypermethrin: A comparative study of their toxicity to the fathead minnow *Pimephales promelas* (Rafinesque) DAR	N	BASF
Varshneya C., Singh T., Sharma L.D., Bahba H.S., Garg S.K.	1992	Immunotoxic responses of cypermethrin, a synthetic pyrethroid insecticide in rats. Short communication. Indian J. Physiol. Pharmacol. 36: 123*126.		
Weiping Liu, Jay J Gan, Sangjin Lee Ingeborg Werner	2004	Isomer selectivity in aquatic toxicity and biodegradation of cypermethrin.	N	
Weiping Liu., Jay J. Gan, Sujie Qin	2005	Separation and aquatic toxicity of enantiomers of synthetic pyrethroid insecticides. *Chirality 17:S127-S133, 2005.* © 2005 Wiley-Liss, Inc.	N	
WHO	1989	Cypermethrin (EHC 82, 1989); International Programme on Chemical Safety, Environmental Health Criteria 82 – Cypermethrin		
_____.	1980	The Toxicity of Cypermethrin to Fathead Minnow (Pimephales promelas) Embryos and Larvae,		
Yousef M.I., El-Deerdash F.M., Kamel K.I., Al-Salhen K.S	2003a	Changes in some hematological and biochemical indices of rabbits induced by isoflavones and cypermethrin. Toxicology 189: 223-234		

8 ANNEXES

Confidential annex