Document II-A

Effects Assessment for the Active Substance

CYPERMETHRIN

CAS No. 52315-07-8
from Arysta LifeScience Benelux sprl
for use in Wood Preservatives (Product Type 8)
and Insecticide (Product Type 18)

Contents

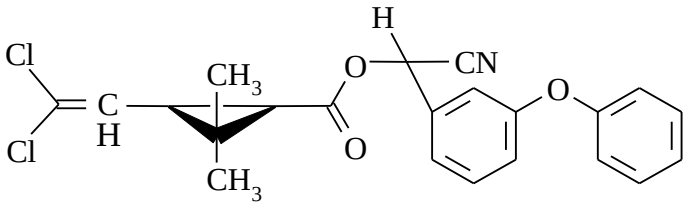
Document II-A – Effects Assessment for the Active Substance

1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTIFICATION OF THE SUBSTANCE

The identification of the active substance Cypermethrin is given in **Table 1.1.**

Table 1.1. Identification of the active substance Cypermethrin

CAS-No.	52315-07-8
EINECS-No.	*257-842-9*
CIPAC-No.	332
IUPAC Name	(RS)-α-cyano-3 phenoxybenzyl-(1RS)-cis, trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate (4 isomer pairs: *cis*-1, *cis*-2, *trans*-3, *trans*-4)
C.A. Name	Cyano(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane carboxylate
Common name	Cypermethrin
Molecular formula	$C_{22}H_{19}Cl_2NO_3$
Structural formula	
Molecular weight (g/mol)	416.3

Additionnal information regarding cypemethrine identification is available in the confidential annex folder.

Isomeric composition

Cypermethrin isomer ratio 40% ≤ cis isomers ≤ 60%

The Cypermethrin molecule has 3 chiral centres giving rise to 8 stereoisomers, four pairs of enantiomers – two cis (CIS 1 & CIS 2) and two trans (TRANS 1 & TRANS 2). Each enantiomeric pair is racemic – i.e. 50:50 mix of each enantiomer.

See Table 1.2 and 1.3 and Fig. 1.1 below.

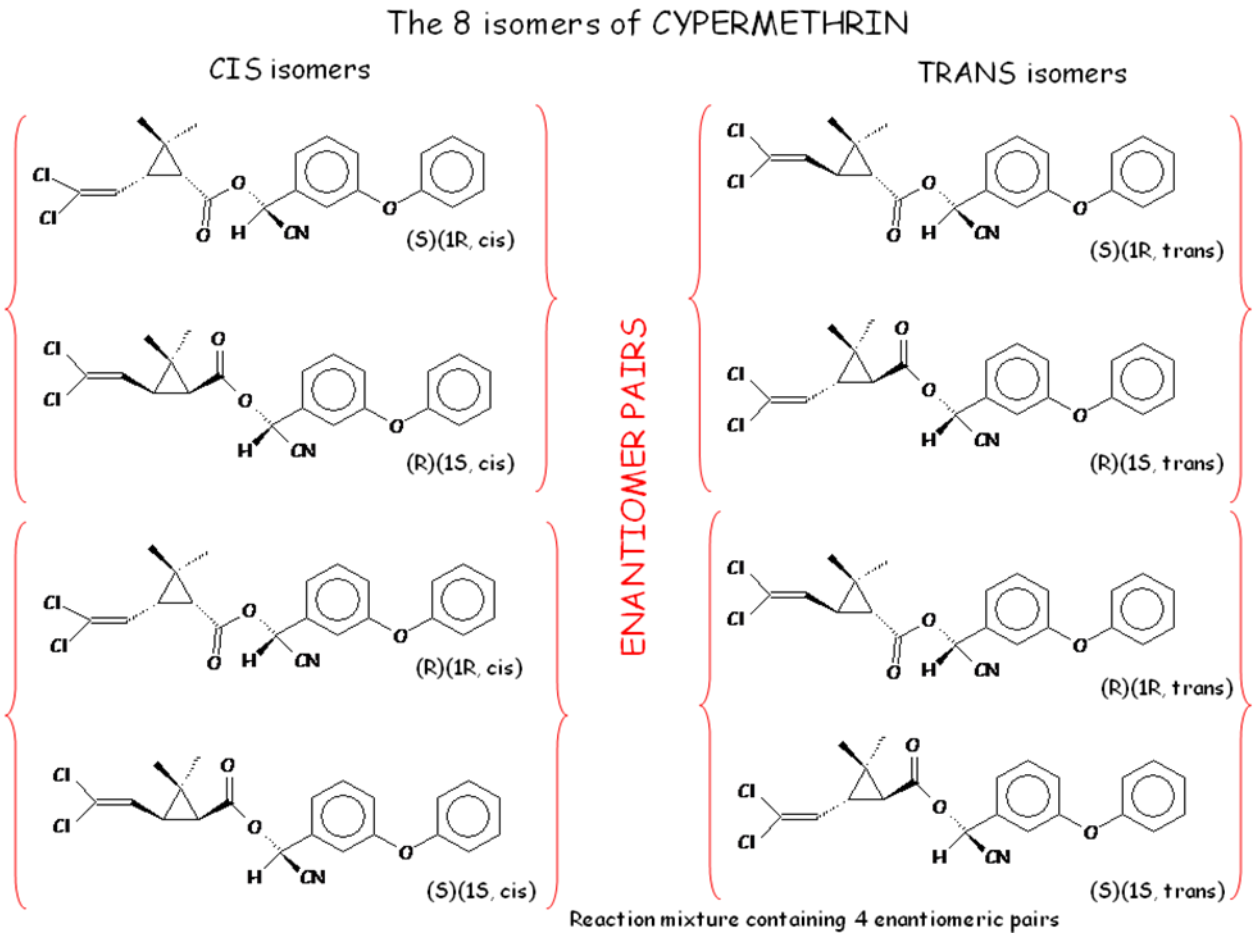
Table 1.2 Overview of the eight isomers of cypermethrin

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

Table 1.3: Cis:Trans Isomer ratios of a typical production batch (no. SL25163S63) of technical cypermethrin (see doc IV_A3.3.1, Bates 2005)

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%

Figure 1.1: 8 Isomers of cypermethrin



It is not possible to further analyse all isomers. Therfore the applicant do not include ratios for the individual isomers in their cypermethrin specification and only refers to the total -cys and –trans isomers (40:60). Representative's batch analysis performed under GLP provided, confirms the isomeric composition of cypermethrin as produced. This is reflected in this competent authority report in which we will further refert to cypermethrin *cis:trans*/40:60. This specification covers all the most recent studies performed during 2005 and later.

1.2 PURITY/IMPURITIES, ADDITIVES IN THE ACTIVE SUBSTANCE

The information contained in tis chapter is considered confidential. Please refers to DocIIA, confidential annex .doc

1.3 PHYSICO-CHEMICAL PROPERTIES OF THE ACTIVE SUBSTANCE

A summary of the physico-chemical properties of Cypermethrin *cis:trans*/40:60 presented under Doc III-A3.1-A3.17 is given in Table 1.3.

Purified cypermethrin *cis:trans*/40:60 (> or = 98% pure) is a white powder with a mild, chemical odour. Cypermethrin *cis:trans*/40:60 as manufactured (> or = 92% pure) is a yellow to brown viscous liquid/semi solid with a mild, chemical odour. Melting point starts at 41.2°C and peaks at 47.3°C (OECD 102). Boiling does not occur, but a decomposition exotherm starting at approx. 200°C is observed (EC A.2.). Its relative density is 1.303 (OECD 109). Its vapour pressure at room temperature is low (2.3 x 10^{-7} Pa at 20°C). Henry's Law Constant is 0.024Pa.m^3.mol^{-1}fraction. Its solubility in water is low. Double distilled water at pH6 gives a solubility <9µg/L at 20°C. It is highly soluble in methanol (248g/L) and heptane (57g/L) both at 20°C. Cypermethrin *cis:trans*/40:60 eluted as 4 discrete components corresponds to log Kow values ranging from 5.3-5.6. Its visocisity is >40000mPa.s at 20°C and 1700 mPa.s at 40°C (OECD 114).

Cypermethrin *cis:trans*/40:60 does not dissociate.

Cypermethrin *cis:trans*/40:60 is thermally stable at room temperature with no decomposition or transformation below 150°C.

Cypermethrin *cis:trans*/40:60 is not flammable. Auto-ignition temperature is 400°C and no flash point was observed up to 110°C. It has no oxidising properties and no potential for explosion (oxygen balance is -194.1).

UV/Vis, IR, proton-NMR and MS give absorption spectra consistent with the structure.

Table 1.3. Physico-chemical properties of Cypermethrin *cis:trans*/40:60

Parameter	Result
Melting point	onset 41.2°C – peak 47.3°C (OECD 102).
Boiling point	boiling did not occur, but a decomposition exotherm was observed, starting at approx. 200°C ().(EC A.2.)
Relative density	D420=1.303 (- EC A.3).
Vapour Pressure	2.3x10-7 Pa at 25°C.
Henry's Law Constant	0.024Pa.m².mol^{-1}fraction
Appearance	Purified a.s.(> or = 98% pure) is a white powder with a mild, chemical odour. The a.s. as manufactured (> or = 92% pure) is a yellow to brown viscous liquid/semi solid with a mild, chemical odour.
Absorption spectra:	UV/Vis, IR, proton-NMR and MS all gave spectra consistent with structure
Solubility in water	Double distilled water (pH6) : <9µg/L at 20°C. (4µg/L used for ecotox assessment)
Dissociation constant	Not applicable, product has very low solubility in water
Solubility in organic solvents	Methanol 248g/L, heptane 57g/L both at 20°C
Partition coefficient n-octanol /water	Cypermethrin eluted as 4 discrete components with retention times corresponding to log Kow values ranging from 5.3-5.6 (EC A.8)
Thermal stability	Thermally stable at room temperature with no decomposition or transformation below 150°C.
Flammability	Not flammable
Auto-ignition temperature	400°C.
Flash point	No flash point up to 110°C
Surface tension	Not applicable as solubility is <1mg/L (OECD 115)
Viscosity	>40000mPa.s at 20°C and 1700 mPa.s at 40°C (OECD 114)
Explosive properties	No potential for explosion. (Oxygen balance is -194.1)
Oxidising properties	Not oxidising.

1.4 ANALYTICAL METHODS FOR DETECTION AND IDENTIFICATION

1.4.1 Analysis of active substance as manufactured

A HPLC method had been developed and validated for the detection of Cypermethrin *cis:trans*/40:60 and Impurities. A GC method has been developed and validated for named impurities (see confidential annexes for further details (Bates, 2002, Covance report no 40/029-D2149 CYP/C66, Covance 40/54 and Covance 40/57).

Table 1.4.1.1 HPLC conditions for samples and standards

HPLC system	Jasco
Column	Spherisorb S5W, 250 x 4.6mm
Column temperature	30°C
Mobile phase	Hexane/1,4-dioxane, 99:1 v/v
Flow rate	1.0ml/min
Wavelength	280nm
Injector volume	50µl
Retention times	approximately13.5min Cis I, approximately 15min Cis II, approximately 16.5min Trans I, approximately 18min Trans II *

**please refers to confidential annex for extra information*

Table 1.4.1.2 GC-FID conditions for analysing impurities not analysed by HPLC.

System:	Perkin-Elmer Autosystems XL
Injector:	Split (approx. 2:1) at 250°C
Sample size:	1µl
Column:	DB1, film thickness 1.5µ m, 15m x 0.53mm
Temperature program:	Initial 45°C Hold 2min 30°C/min to 200°C 5°C/min to 290°C 20°C/min to 310°C Final hold 10min
Carrier gas	Helium at 10ml/min
Detector:	FID at 260°C
Gases:	Hydrogen at approx. 50ml/min Air at approx 500ml/min
Retention times	Confidential*

**please refers to confidential annex for extra information*

1.4.2 Residue analysis

Analytical methods for the determination of residues of Cypermethrin *cis:trans*/40:60 and relevant metabolites are given in Table 1.4.3.

Table 1.4.3. Analytical methods for the determination of residues of Cypermethrin and relevant metabolites

Sample	Test substance	Analytical method	Fortification range / Number of measurements	Linearity	Specificity	Recovery rate (%)			Limit of determination (LOQ)	Reference
						Range	Mean	Relative standard deviation		
Soil and Sediment	Cypermethrin 40:60 cis:trans	GC/MS on soil fortified with known amounts of cypermethrin	0.05mg/Kg and 0.5mg/Kg in soil 0.5µg/Kg and 5.0µg/Kg for sediment.	Linear over range 0.005 to 1.0 µg/ml. Coefficient of determination (r^2) were ≥0.98 and linearity was therefore established.	Specific	Soil: Between 95-107% for each stereoisomer and for total cypermethrin. Sediment: Between 70-110% apart from two isomers, trans I (65-87%) and trans II (97-128%).			LOQ = 0.05mg/Kg for cypermethrin (total isomers) in soil. LOQ = 0.5µg/Kg for cypermethrin (total isomers) in sediment.	Wimbush, 2003, Covance study 40/039
Air	Cypermethrin 40:60 cis:trans	GC-MS on air fortified with known amounts of cypermethrin	Equiv to 0.375µg/m^3 (LOQ) and 3.75µg/$m^{3.}$	Non-linear over range 0.01 to 0.3µg/ml (total cypermethrin), with coefficients of determination (r^2) ≥0.98	Specific	93% ambient air 87% elevated (35°)			LOQ=0.375µg/m^3	Wimbush 2005,Covance study 1669/016vance study 1669/016
Surface water	Cypermethrin 40:60 cis:trans	GC/ECD on water fortified with known amounts of cypermethrin	0.01 and 0.1µl.	Linear over range 0.005 to 0.5µg/ml. Coefficient of determination was ≥0.99.	Specific	All within 70-110% apart from trans II (103-112%)			LOQ=0.01µg/L	Wimbush 2002, Covance study 40/040

Sample	Test substance	Analytical method	Fortification range / Number of measurements	Linearity	Specificity	Recovery rate (%)			Limit of determination (LOQ)	Reference
						Range	Mean	Relative standard deviation		
Products of animal origin	cypermethrin *cis:trans*/40:60	capillary GC with MSD, with chemical ionisation in the negative mode An independent laboratory validation of the analytical methods in products of animal origin is provided.	bovine tissue: 0.05-0.50 mg/Kg bovine milk: 0.005-0.050 mg/Kg hen eggs: 0.01-0.10 mg/Kg n=5	Linear over range 0.01 to 1.0 µg/ml (total cypermethrin). 6 standard solutions of increasing cypermethrin concentration Coefficient of determination $r^2 \geq 0.98$	no interference > 30% of LOQ in the control matrices	Mean recovery between 70% and 110% Analysis of each validation level in quintuplicate RSD ≤ 20% for each stereoisomer and total cypermethrin			bovine tissues: LOQ= 0.05 mg/Kg bovine milk: LOQ= 0.005 mg/Kg hen eggs: LOQ= 0.01 mg/Kg	Wimbush, 2003a. Covance study 40/041

Sample	Test substance	Analytical method	Fortification range / Number of measurements	Linearity	Specificity	Recovery rate (%)			Limit of determination (LOQ)	Reference
						Range	Mean	Relative standard deviation		
Products of plant origin	cypermethrin *cis:trans*/40:60	DFG multi-residue method S23: capillary GS with ECD. Confirmation: column of different polarity. An independent laboratory validation of the analytical methods in products of plant origin is provided.	oilseed rape (seed and oil): 0.05-0.5 mg/Kg wheat (grain and straw): 0.025-0.25 mg/Kg n=5	Linear over range 0.02 to 1.5 µg/ml (total cypermethrin), n>6. Coefficient of determination $r^2 \geq 0.98$	no interference > 30% of LOQ in the control matrices	Mean recovery between 70% and 110%, with the exception of the cis-I, trans-I, trans-II isomers in wheat straw at 0.025 mg/Kg (recoveries of 125%, 114%, 115%, respectively). Overall recovery for total cypermethrin in wheat straw was acceptable at 0.025 mg/Kg (110%). Confirmation analysis gave mean recoveries within the acceptable range for each stereoisomer and for total cypermethrin. Analysis of each validation level in quintuplicate RSD ≤ 20% for each stereoisomer and total cypermethrin			oilseed rape: LOQ= 0.05 mg/Kg wheat: LOQ= 0.025 mg/Kg	Wimbush, 2003b. Covance study 40/037

1.5 CLASSIFICATION AND LABELLING OF THE ACTIVE SUBSTANCE

1.5.1 Current classification Cypermethrin

The classification of Cypermethrin *cis:trans/40:60* was agreed at the 29th ATP and appears in Annex I of former Directive 67/548/EEC containing the list of harmonised classifications and labelling for substances.

Currently, , Cypermethrin *cis:trans/40:60* has a harmonised classification as listed in Annex VI table 3.1. to Regulation (EC) No 1272/2008.

Classification	**as in Directive 67/548/EEC (29th ATP)**	
Class of danger	Xn:	Harmful
	N:	Dangerous for the environment
R phrases	R20:	Harmful by inhalation
	R22:	Harmful if swallowed
	R37:	Irritating to respiratory system
	R50/R53:	Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment
S phrases	S 2:	Keep out of the reach of children
	S36/37/39:	Wear suitable protective clothing, gloves and eye/face protection
	S60:	This material and its container must be disposed of as hazardous waste
	S61:	Avoid release to the environment

Classification	**as in Regulation EU CLP 1272/2008**	
GHS Pictograms	GHS07 GHS09	
Signal Word	Warning	
Hazard Class and Category Codes	Acute Tox. 4 Acute Tox. 4 STOT SE3 Aquatic acute 1 Aquatic chronic 1	
Hazard Statement Codes	H332 H302 H335 H400 H410	Harmful if inhaled harmful if swallowed May cause respiratory irritation Very toxic to aquatic life Very toxic to aquatic life with long lasting effects
Precautionary Statement Codes	P261 P314 P273 P391 P501	Avoid breathing vapours/spray Get medical advice/attendtion if you feel unwell Avoid release to the environment Collect spillage Dispose of content in accordance with local/national regulation

1.5.2 Proposed classification of Cypermethrin

On the basis of a review of the data submitted by the applicant and additional open literature data, the BE CA conclude that the current classification of Cypermethrin *cis:trans*/40:60 on Annex I to Directive 67/548/EEC could be maintained.

No new scientific information/data is available that may affect the classification of the active substance. Nevertheless, in CLP-Regulation (EC) No 1272/2008 the guidance values are modified for 'specific target organ toxicity following repeated exposure'. Because of the change in guidance values, the clinical effects of neurotoxicity observed in both animals and humans, and the liver toxicity observed in animals, new **classification/Labelling of the active substance 'cypermethrin** *cis:trans/40:60***'** for repeated-dose toxicity according to the criteria (modified guidance values) in CLP-Regulation (EC) No 1272/2008 2nd ATP is justified: **STOT RE2; H373. May cause damage to organs through prolonged or repeated exposure.**

The proposal has still to be validated by ECHA.

Classification	**proposed by the BE CA (EU CLP regulation 1272/2008 2nd ATP)**
GHS Pictograms	GHS08 GHS09
Signal Word	Warning
Hazard Class and Category Codes	Acute Tox. 4 STOT RE2 STOT SE3 Aquatic acute 1 M = 100 Aquatic chronic 1 M= 1000
Hazard Statement Codes	H332 Harmful if inhaled H302 harmful if swallowed H373 May cause damage to organs through prolonged or repeated exposure H335 May cause respiratory irritation H400 Very toxic to aquatic life H410 Very toxic to aquatic life with long lasting effects

2 EFFECTIVENESS AGAINST TARGET ORGANISMS

2.1 FUNCTION

Cypermethrin is an insecticide for use in pest control (product type 18 of the EU Biocidal Products Directive).

2.2 FIELD OF USE ENVISAGED

Control of crawling and flying insect pests in and around domestic and public buildings, including farm buidings, animal housing and food processing factories.

Products containing CYPERMETHRIN (in spray formulations) are intended to be used by professionals (Pest Control Operators) as a broad spectrum insecticide against crawling and flying insects, including cockroaches, ants, fleas, bedbugs, flies, mosquitoes, moths and wasps nests for mainly indoor use as a surface spray on walls and floors and also for outdoor use on paths and patios and around the edges of buildings.

2.3 EFFECTS ON TARGET ORGANISMS

2.3.1 Efficacy

For the purpose of Annex I entry, the applicant has provided several studies on the efficacy of CYPERMETHRIN against house flies, German and Oriental cockroaches, garden ants and cat fleas.

As all the studies were performed on formulated products all the study summaries and the summary table of the studies are in Doc IIB.

2.3.2 Development of resistance.

Information on resistance to CYPERMETHRIN, and the potential for resistance to develop following the use of CYPERMETHRIN, is presented in the BE CA Report, Document II-B, Section 2.5.

3 HUMAN HEALTH EFFECTS ASSESSMENT

In this Section, summaries and evaluation of data presented in Doc.III-A6, Toxicological and metabolic studies of the CA-Report are reported as far as possible in summary tables. This data were discussed and approved for the inclusion of cypermethrin in the list a of approved active substance for the PT 8. Since no new data were provided for the PT 18 evaluation, this section has not been revised. However, as agreed during the BPC Working Group IV 2016, a DNT study will be added in the CAR no later than six month before inclusion in order to address the concern raised during the evaluation by EFSA of another cypemethrin for the reference value.

The data highlighted by the use of a grey background in the tables are from studies where a full (robust) **STUDY SUMMARY** made in accordance with the Technical Notes for Guidance on Dossier Preparation was available, i.e. the **KEY STUDIES.** Summaries of the rest of the studies are available as **IUCLID** entries only.

The data provided fulfil the core and additional data requirements for both the active substance and the representative biocidal product for the field of use applied for.

Unless otherwise stated, all studies were made according to internationally accepted guidelines and principles for Good Laboratory Practice (GLP).

The allocation of reliability scores 1 or 2 to the Key Studies indicates that results from such studies can be considered for risk assessment. Supplementary studies (i.e. studies with a reliability score of 3 or more, or even 0) give additional information for the risk assessment and are available as IUCLID entries.

For every toxicological end-point a proposal for classification/labelling according to the criteria in Directive 67/548/EEC is given.

Cypermethrin possesses three chiral carbon atoms and is therefore a racemic mixture of 8 isomers (four *cis*- and 4 *trans*-isomers). The technical products commonly available contain more than 92% cypermethrin and the ratio *cis*- to *trans*-isomers varies from 50/50 to 40/60.

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: α S configuration of the α-cyano carbon is a potent mammalian toxicant, whereas the α-R enantiomers are essentially non-toxic.

Thus, the more active components of cypermethrin are 1R cis α S and 1R trans α S, e.g. approximately 25% of the mixture. Less active isomers are 1R cis α R; 1S cis α S ; 1R trans α R and 1S trans α S e.g. approximately 50% of the mixture . Relatively non-active isomers are 1S cis α R and 1 S trans α R e.g. approximately 25% of the mixture.

Nontheless, it has been shown that trans-isomer are metabolised more rapidely than the cis isomer which may be the cause of the relatively higher toxicity of the *cis*-isomer in mammal compared to the *trans*-isomer.

In relation with cypermethrin, literature sometimes reveals contradictory results. These can be related to the different animal strains, doses, administration route, vehicle, treatment schedules used, but can also be attributed to the different purity and *cis:trans* ratio of the cypermethrin used.

3.1 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

Key studies: The toxicokinetic properties of cypermethrin have been investigated in the rat after oral administration, and dermal absorption has been investigated in an *in vitro* study with human skin and an *in vivo* rat study.

Additional information was found in the open literature. The summaries of these studies are available as IUCLID entries.

Table 3.1-2 Summary of key ADME studies and studies relevant for determination of the oral and dermal absorption

Route	Species	Test substance	Dose (mg/Kg bw)	Method	Results	Reference
Oral	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, 144 h; 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	<u>Excretion Balance study</u> *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 plus cage washes and debris, was 52.8% of the dose in the males and 57.6% in the females. The residual carcass contained <0.7% of the dose showing that elimination of the radioactive dose was complete. *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 plus cage washes and debris, was 51.3% of the dose in the males and 43.6% in the females. The residual carcass contained 0.4 - 0.6% of the dose showing that elimination of the radioactive dose was essentially complete. *High dose, 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	[redacted] DocIIIA6.2 (01)

urine of the females, though, there were significant inter-individual variations in the route of excretion. However, the main route of excretion was *via* the faeces (78.6 and 60.7% of the dosed radioactivity in males and females respectively), the urine contained a further 27.2% of the dose in the males and 36.9% in the females. There was no significant elimination of [^{14}C]-carbon dioxide in the expired air (<0.2% of the dose). The minimum absorption, as measured by the radioactivity excreted in the urine plus cage washes and debris, had fallen at the high dose to 28.7% of the dose in the males and 42.5% in the females. The 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 plus cage washes and debris, was 31.5% of the dose in the males and 38.4% in the females. The residual carcass contained 0.5% of the dose showing that elimination of the radioactive dose was essentially complete.

In conclusion, oral absorption:

	Low dose 3 mg/Kg bw			High dose 50 mg/Kg bw	
group	♂	♀		♂	♀
A	52.8%	57.6%	B	28.7%	42.5%
C	51.3%	43.6%	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 24 h after nine doses (565.5 ng equivalents/g) were twice those seen 24 h after a single oral dose. The highest increases (>10-fold) in the concentration of radioactivity were measured in the inguinal and peri-renal fat. In these tissues, the concentrations of residues rose from 91.8 to 1009 ng equivalents/g in the case of the inguinal fat and from 197.5 to 1966 ng equivalents/g in the case of the peri-renal fat. The lowest levels of radioactivity were seen in the brain (<9 ng equivalents/g) and spinal cord (<36 ng equivalents/g). In female rats, the levels of radioactivity in the plasma were approximately 20% higher on Day 10 (698.2 ng equivalents/g) than on Day 2 (579.5 ng equivalents/g). The levels in the inguinal and peri-renal fat rose by 6-7 times those seen on Day 2, the concentrations of residues rising from 204 to 1196 ng equivalents/g in the case of the inguinal fat and from 295 to 2179 ng equivalents/g in the case of the peri-renal fat. The lowest levels of radioactivity were seen in the brain and spinal cord (<21 ng equivalents/g). The radioactivity in the tissues was rapidly cleared, and by Day 16, 7 days after the last dose, many of the tissues contained levels of radioactivity that had fallen below the limit of detection. The concentrations of radioactivity in the fats had fallen by 2-6 times when compared to the levels on Day 10 whilst the levels in the plasma had fallen by approximately 30 times.	

Oral	Rat female n=60 3/group	**Cypermethrin *cis:trans*/50:50** ^{14}C-benzyl-labelled ^{14}C-cyclopropyl-labelled (only biotransformation study)	Biotransformation study : single dose, 200 mg/Kg bw Bioaccumulation and elimination study: 2 mg/Kg bw/d in maize oil, 10 weeks	Biotransformation study Bioaccumulation elimination study Tissues: liver, kidney, adipose tissue, blood (whole blood and plasma), skin, ovaries	<u>Biotransformation study</u> Elimination of radioactivity via urine and faeces, expressed as the percentage of the dose administrered (200 mg/Kg bw): Urine ♂ 29 ♀ 33 Faeces ♂ 55 ♀ 59 ^{14}C-benzyl-label ^{14}C-cyclopropyl-label 41 56 46 34 The administered radioactivity was excreted within 7 days in both urine and faeces. The major pathways of metabolism involved cleavage of the ester bond, with subsequent hydrolysation and glucuronidation of the cyclopropyl acid moieties, together with hydroxylation and sulphation of the 3-phenoxybenzyl moiety. The absence of sex-and ose-dependent changes was reflected by the constant proportions of these metabolites found in the urine. <u>Bioaccumulation and elimination study</u> Levels in all tissues reached a plateau after 56 days of dosing. The extent of accumulation, expressed as mg equivalents of cypermethrin per Kg tissue, was: fat, 3.91; liver, 0.97; kidneys, 0.69; ovaries, 0.03; skin, 1.89; whole blood, 0.35; and plasma 0.64. Analysis of fat samples, 24 h after the final dose, revealed that the rate of elimination of radioactivity from fat was biphasic in nature. In fat the *cis* isomer represented 88% of the residu, the *trans* isomer 12%. The *cis* and *trans* isomers were eliminated with elimination half-lives of 18.24 and 3.43 days respectively to reach bacKground levels 7 weeks after stop of dosing. Levels of 14C residues in the liver, kidneys, and blood reached control bacKground levels within 29, 8, and 15 days, respectively, of the final dose. Apart from fat, the only other tissue that contained radioactivity was the skin; the rate of elimination of radioactivity from the skin was similar to that for fat. Accumulation in the sciatic nerve was also studied in rats dosed for 26 days. No appreciable bioaccumulation was found to occur in the sciatic nerve.	[redacted] DocIIIA6.2 (03)
Oral	Rat	**Cypermethrin**	1 to 5 mg/Kg bw,	Balance and tissue residue	Elimination	[redacted]

	m/f Balance and tissue residue studie: 6/sex/group Fat residue depletion study: 2 f/group Biliary elimination study: 2 males	NRDC 149 ^{14}C-benzyl-labelled, ^{14}C-cyclopropyl-labelled, ^{14}C-cyano-labelled	single dose	study; Fat residue depletion study; Biliary elimination study	Radioactivity derived from the benzyl-^{14}C and cyclopropyl-^{14}C labeling was rapidly eliminated, mostly in the urine. Results: 3 rats per sex given single oral doses of cyclopropyl-^{14}C cypermethrin (1.2-2.2 mg/Kg) and sacrificed 3 days after dosing. Results expressed as percentage of given dose: Urine ♂ ♀ Faeces ♂ ♀ ^{14}C-cyclopropyl-label 66.5 55.8 27.0 28.7 Tissue residues (benzyl-^{14}C label) Residues derived from cis-cypermethrin were measured 1, 3, and 8 days after a single oral dose. One day after dosing, residual radioactivity lay in the order of fat>liver>kidney>blood>muscle>brain. Tissue residues were generally low (0.01 µg/g in brain) with the exception of fat (~1µg/g); *cis*-isomer residues being higher than *trans*-isomer residues. Benzyl-^{14}C-*cis*-cypermethrin residue elimination was rapid (half-life < 1 day) from all tissues except for fat (half-life: 11.7 days). The residue in the fat was largely due to unchanged *cis*-cypermethrin Compared with the urinary route, only a very small proportion of orally administered [cyclopropyl-^{14}C]cypermethrin (metabolites) was eliminated in the bile. The use of beta-glucuronidase treatment and TCL analysis indicated that the radioactivity was due to the glucuronide conjugates of the cis- and trans-cyclopropanecarboxylic acid derived form the hydrolysis of cypermethrin.	[redacted] DocIIIA6.2 (03)
Oral	Mice male 2/group (^{14}C-benzyl labelled)	**Cypermethrin *cis or trans*** ^{14}C-benzyl-labelled, ^{14}C-cyclopropyl-	Single dose 8 mg/Kg bw (^{14}C-benzyl labelled) 7 mg/Kg bw (^{14}C-cyclopropyl-labelled)	Metabolism study	Elimination: Radioactivity was rapidly and almost completely eliminated in the urine and faeces of mice following single oral doses of cis- or trans-cypermethrin with either ^{14}C-benzyl or ^{14}C-cyclopropyl labelling. The urine was the major route of	[redacted] DocIIIA6.2 (03)

	3/group(^{14}C-cyclopropyl-labelled)	labelled			elimination for trans-cypermethrin metabolites (66-80%) whereas it was less important for those of cis-cypermethrin (31-41%) compared with the faeces. Metabolism: Metabolism occurred mainly via ester cleavage and elimination of the *cis*- and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylic acid moieties as the glucuronide conjugates. The α-cyano-3-phenoxybenzyl alcohol released as a results of ester cleavage was mainly converted to 3-phenoxybenzoic acid which was partly eliminated unchanged, partly conjugated with amino acids (mainly taurine) and glucuronic acid and partly oxidised to 3-(4-hydroxyphenoxy) benzoic acid which was then excreted as the sulphate conjugate.	
Oral	Human volunteers males, n=6	**Cypermethrin *cis:trans*/50:50**	Single dose 38 mg/ml in ethanol on a sugar cube,followed by 200 ml water = 3.3 mg	Metabolism Collection of urine samples over the periods 0-4, 4-8, 8-12h post treatment, then over 12h intervals up to 120 hrs after dosing Urinary metabolites DCVA, 3PBA, 4OH4PBA, 2OH3PBA were analysed	Absorption of Cypermethrin was rapid and peak excretion rates were seen in the first 4 hours after dosing for the hydrolysis products – *cis* and *trans* DCVA. For the oxidised metabolites 3PBA and 4OH3PBA, the peak rates were seen between 4 and 24 hours after dosing. On average, 93% of recovered metabolites were excreted within the first 72 hours after dosing. For the majority of individual volunteers, some or all of the metabolites were still detectable in urine at five days after dosing, although the concentrations were approaching the limit of detection. Excretion rates for all four metabolites were similar when individual volunteer data were assessed. The elimination half life for total metabolites was 16.5 hours. The *trans/cis* DCVA ratio was 2:1 on average and the total amounts of recovered DCVA and total phenoxybenzoic acid in urine were similar. The absorbed proportion of administered dose was estimated based on the total recovery of trans DCVA – mean 36% (range of estimate 27-57%).	Woollen et al., 1992 DocIIIA6.2 (04)
Oral	Human volunteers n=4	**Cypermethrin *cis:trans*/50:50**	Single dose (n=2) 0.25 mg, 0.5 mg (in corn oil, in gelatin	Dose-excretion study	Twenty-four hour urine samples were collected up to a period of 5 days. After a single oral dose, urinary excretion was rapid and confined to the first 24 h sample: 78% of the *trans* isomer	Eadsforth and Baldwin,

			capsule) Repeated dose (n=2) 1.0 mg and 1.5 mg separated by 12 months; 0.25 mg and 1.0 mg separated by 12 months		and 49% of the *cis* isomer as free and conjugated cyclopropane carboxylic acid. This confirms that, as in other mammals, ester cleavage and elimination of the *cis* and *trans* cyclopropanecarboxylic acid moieties in the free and conjugated form is a major route of metabolism of cypermethrin in man.	1983 DocIIIA6.2 (03)
Dermal	Rat males 4/group	**^{14}C-Cypermethrin *cis:trans*/40:60**	500 g/L 25 mg/L	OECD 427	Dermal absorption: Radioactivity absorption (%) at 216h 500 g/L 25mg/L Absorbed: 3.44 11.03 + residual skin: 4.77 11.2 +18 tape strips: 6.7 12.7	[redacted] DocIIIA6.2 (05)
Dermal	In vitro Human skin 4 donors	**^{14}C-Cypermethrin *cis:trans*/40:60**	25 mg/L (0.00025 mg/cm²) 100 g/L (1.0 mg/cm²) 8h exposure	OECD 428 Static cell system The membranes were not occluded and exposed for 8 hours, then the skin was washed. Receptor fluid collected up to 24h post dose. At 24h post dose the skin was tape stripped.	Dermal absorption: Absorption was rapid with detectable levels in the receptor fluid at 1 h. Radioactivity absorption (%) 100 g/L 25mg/L Receptor fluid: 1.5 13 + residual skin: 27 68.6 +5 tape strips: 37.5 78.6 Conclusion on dermal absorption: concentrate (100 g/L): 37.5% dermal absorption	Hardwick, 2006 DocIIIA6.2 (02)

					spray dilution (25 mg/L): 78.6% dermal absorption	
Dermal	Human volunteers males, n=6	**Cypermethrin *cis:trans*/56:44**	Single dose 26 mg/ml in soya bean oil on 800 cm^2 = 31 mg	Metabolism Collection of urine samples over the periods 0-4, 4-8, 8-12h post treatment, then over 12h intervals up to 120 hrs after dosing Urinary metabolites DCVA, 3PBA, 4OH4PBA, 2OH3PBA were analysed	41% of the dermal dose (range 36-48%) was recovered in the detergent skin wash after the completion of the 8 hour exposure period. Extracts from the T-shirt cover used overnight post exposure produced a further 24% of applied dose. On average in this study at least 65% of the applied dose was not absorbed. Cypermethrin metabolites were detectable in the majority of urine samples over the first four hours of exposure but peak excretion rates occurred between 12 and 36 hours post dosing. No metabolites were detected beyond the 96 h sampling point (except for trace amounts of 4OH3PBA in two individuals). The elimination half life for total metabolites was 13 h (range 8-22h, standard deviation ±5.1 h). Four individual volunteers took part in both the oral and dermal phases of the assay, these individuals had similar elimination half lives for both exposure routes. The average *trans:cis* DCVA ratio was 1:1.2. The amounts of cyclopropane acid metabolites in urine samples following dermal application were circa four times lower than the metabolites derived from the phenoxybenzyl moiety. The estimate of Cypermethrin dermal absorption, based on *cis* or *trans* DCVA metabolite presence, was 0.3%, this was much lower than the same estimate based on 3PBA and 4OH3PBA – mean of 1.2% dermal absorption estimated.	Woollen et al., 1992 DocIIIA6.2 (04)

ORAL

The absorption, distribution and excretion of cypermethrin was investigated according to OECD guideline 417 in a recent study ([redacted]). Male and female rats were given a single oral dose of 3 or 50 mg [14C]-cypermethrin cis:trans/40:60 labelled in either the cyclopropyl or phenyl ring and the rates and routes of excretion of the radioactivity were determined. A separate group of rats received up to 9 daily oral doses of 3 mg [14C-phenyl]-cypermethrin cis:trans/40:60 /Kg bw and the concentration of radioactivity was determined in the tissues at 24 h after 1, 7 and 9 doses and at 7 days after 9 doses.

3.1.1 Absorption

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

At the low dose 51.3 to 52.8% of the dose was absorbed by the male rats and 43.6 to 57.6% in case of the females, as measured by the total radioactivity in urine and cage washes. At the high dose, 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.

The oral absorption data derived from the [redacted] study is in agreement with the data from the older published literature.

The elimination of radioactivity after a single oral administration of cypermethrin cis:trans/50:50 of 200 mg/Kg bw, in male and female rats was excreted within 7 days in both urine (^{14}C-benzyl-label: 29% and 33%; ^{14}C-cyclopropyl-label: 41% and 56%) and faeces (^{14}C-benzyl-label: 55% and 59%; ^{14}C-cyclopropyl-label: 46% and 34%) as determined in [redacted].

Radioactivity derived from the benzyl-^{14}C and cyclopropyl-^{14}C labeling was rapidly eliminated, mostly in the urine following a single oral dose of ^{14}C-cypermethrin, 1.2-2.2 mg/Kg bw, in male and female rats and sacrificed 3 days after dosing ([redacted]). The radioactivity derived from the cyclopropyl-^{14}C labeling was eliminated mainly in the urine of both males and females (^{14}C-cyclopropyl-label: 66.5 and 55.8%), the remainder was eliminated in the faeces (^{14}C-cyclopropyl-label: 27.0 and 28.7%).

In addition, there is published human data available. Cypermethrin was administered orally to 6 male volunteers as a single dose of 3.3 mg (*cis:trans*/1:1) (Woollen et al., 1992). Cypermethrin was dissolved in ethanol to give a final concentration of 38 mg/mL. The single oral dose was administered by adding 86µL of the dose solution to a sugar cube, allowing to air-dry for 20 minutes to allow ethanol to evaporate. The treated cube was swallowed, followed by 200 mL of water. Urine was recovered over the periods 0-4, 4-8, 8-12 h post treatment and then over 12h intervals up to 120 hours after dosing. Total volume, creatinine concentration and pH were analysed in the samples together with linear regression analysis of half-life – assessed on excretion rate versus mid-point time from 18 h post treatment t the point at which metabolites fell below the limit of detection. Urinary metabolites of Cypermethrin [trans 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid = DCVA, 3-phenoxybenzoic acid = 3PBA, 3-(4'-hydroxyphenoxy)benzoic acid = 4OH4PBA) were analysed together with internal standards 4-phenoxybenzoic acid (4PBA) and 4OH4PBA. The limit of detection was typically 0.5µg/L for all three metabolites and the precision is cited in the publication to be typically 5% relative standard deviation. Absorption of Cypermethrin was rapid and peak excretion rates

were seen in the first 4 hours after dosing for the hydrolysis products – *cis* and *trans* DCVA. For the oxidised metabolites 3PBA and 4OH3PBA, the peak rates were seen between 4 and 24 hours after dosing. On average, 93% of recovered metabolites were excreted within the first 72 hours after dosing. For the majority of individual volunteers, some or all of the metabolites were still detectable in urine at five days after dosing, although the concentrations were approaching the limit of detection. Excretion rates for all four metabolites were similar when individual volunteer data were assessed. The elimination half life for total metabolites was 16.5 hours. The *trans/cis* DCVA ratio was 2:1 on average and the total amounts of recovered DCVA and total phenoxybenzoic acid in urine were similar. The absorbed proportion of administered dose was estimated based on the total recovery of trans DCVA – mean 36% (range of estimate 27-57%). Nevertheless, it is difficult to assess the human volunteer data from Woollen et al. (1992) because the mass balance is not reported.

Conclusions on oral absorption

Key study: Needham, 2006 (rat study):

	Low dose 3 mg/Kg bw			High dose 50 mg/Kg bw	
group	♂	♀		♂	♀
A	52.8%	57.6%	B	28.7%	42.5%
C	51.3%	43.6%	D	31.5%	38.4%

Low dose (3 mg/Kg bw): 43.6 to 57.6% (♂ 51.3 to 52.8%, ♀ 43.6 to 57.6%)

High dose (50 mg/Kg bw): ♂ 28.7 to 31.5%, ♀ 38.4 to 42.5%

A, B: ^{14}C-cyclopropyl-labelled; C,D: ^{14}C-phenyl-labelled

Open Literature (rat studies):Elimination of radioactivity via urine, expressed as the percentage of the dose administered:

	Crawford et al., 1981 1.2-2.2 mg/Kg bw		Rhodes et al., 1984 200 mg/Kg bw	
group	♂	♀	♂	♀
^{14}C-cyclopropyl-labelled A	66.5%	55.8%	41%	56%
^{14}C-benzyl-labelled			29%	33%

Open Literature (human studies):

Woollen et al., 1992 (human volunteer):

27 to 57% (3.3 mg on sugar cube, followed by 200 ml water = 0.055 mg/Kg bw), mean = **36%**, median = 30%

For the estimation of oral absorption, a conservative approach is adopted. Different values were adopted for animals and humans.

Animals

Most of the animal studies were performed with a wide range of doses. For AEL setting, the POD for the all AEL (acute, medium-term and long-term) is an oral LOAEL: 5 mg/Kg bw/d.
Because the PODs for the AELs are closer to the low dose (3 mg/Kg bw) rather than the high dose (50 mg/Kg bw) of the Needham study (2006), it is reasonable to use an oral absorption value of **44%** for animals for deriving systemic NOAELs (Agreed at TM II 2011).

Humans

For the estimation of human systemic exposure, an oral absorption value of **57%** is adopted, based on the low dose data from the [redacted] and using a conservative approach. The value of 57% is also consistent with observations in humans (Woollen et al., 1992).

3.1.2 Distribution

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

The high dose rate was 15-17 times greater than the low dose, and the concentration of radioactivity in the tissues did not automatically increase in direct proportion to the dose level. The concentrations of radioactivity in the tissues in rats receiving [^{14}C-cyclopropyl] -cypermethrin, were 7-9 times greater at the high dose than at the low dose for the fat, liver and kidneys and 23 times greater for the adrenals. In the females, the concentrations of radioactivity in the tissues were 6-10 times greater at the high dose for the liver and adrenals, and 14-17 times for the fat, kidney, and ovaries. When the rats were dosed with [^{14}C-phenyl]-cypermethrin, the concentrations 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 were 15 times higher for the liver, 6 times higher for the adrenal, and 15-21 times higher for the kidney, fat and ovaries.

Following repeated daily oral administration of [^{14}C-phenyl]-cypermethrin at a dose level of 3 mg/Kg for up to 9 days, the levels of radioactivity in the tissues increased with the number of doses received. In males, the levels in the plasma 24 h after 9 doses were twice those seen 24 h after a single oral dose. The highest increases 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 above 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.

These findings are in agreement with the older published literature ([redacted]).

In a bioaccumulation elimination study with ^{14}C-benzyl-labelled cypermethrin (*cis:trans*/1:1) dosed 2 mg/Kg bw in maize oil, daily for 10 weeks in rats ([redacted]) the fat was identified as the tissue showing the greatest deposition of cypermethrin and its metabolites (twice the concentration of the plasma). Levels of all tissues reached a plateau after 56 days of dosing. Radioactivity was rapidly eliminated on the cessation of dosing, although less rapidly for fat and skin. The rate of elimination of radioactivity from fat was biphasic in nature. The main components in the fat were *cis*-isomers of cypermethrin which were eliminated with a mean half-life of 18.2 days; the *trans*-isomers were eliminated with a mean half-life of 3.4 days. Background levels were reached 7 weeks after the stop of

dosing. Levels of ^{14}C residues in the liver, kidney, and blood reached control background levels within 29, 8, and 15 days.

Accordingly, *cis*- and *trans*- isomers of cypermethrin have been studied in rats (1-5 mg/Kg) by using 3 forms of radiolabeling (benzyl-^{14}C, cyclopropyl-^{14}C, cyano-^{14}C). Radioactivity derived from the benzyl-^{14}C and cyclopropyl-^{14}C labeling was rapidly eliminated, mostly in the urine. Residues derived from cis-cypermethrin (benzyl-^{14}C label) were measured 1, 3, and 8 days after a single dose. One day after dosing, residual radioactivity lay in the order fat > liver > kidney > blood > muscle > brain. Tissue residues were generally low (0.01 µg/g in brain) with the exception of fat (~1µg/g); *cis*-isomer residues being higher than *trans*-isomer residues. Benzyl-^{14}C-*cis*-cypermethrin residue elimination was rapid (half-life < 1 day) from all tissues except for fat (half-life: 11.7 days). The residue in the fat was largely due to unchanged *cis*-cypermethrin ([redacted]).

3.1.3 Excretion

[redacted] study: Excretion was virtually completed by 72h following a single oral dose of ^{14}C-cyclopropyl- or ^{14}C-phenyl-cypermethrin at a dose of 3 or 50 mg/Kg bw. Urinary and faecal excretion were similar at the low dose for both radiolabels, but at the highest dose level faecal excretion predominated, especially in the males. At 50 mg/Kgbw, faecal excretion was the major route of elimination accounting for 79 and 61% for males and females respectively when [^{14}C-cyclopropyl]-cypermethrin was dosed, and 80 and 68% for males and females respectively when [^{14}C-phenyl]-cypermethrin was dosed. In each case, the higher excretion level was seen in the male rats. The observed increase in faecal elimination suggests that the absorption process was being saturated at 50 mg/Kg bw. At the low dose (3 mg/Kg bw), 51.3 to 52.8%of the dose was absorbed by the male rats and 43.6 to 57.6% in case of the females, as measured by the total radioactivity in urine and cage washes. At the high dose (50 mg/Kg bw), 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. There was no significant elimination of ^{14}C-carbon dioxide in the expired air.

The metabolism of cypermethrin in maize oil was studied in male and female Wistar rats following a single toxic oral dose of 200 mg/Kg bw of 2 radio-labelled forms (^{14}C-benzyl and ^{14}C-cyclopropyl) of cypermethrin ([redacted]). Minimal amounts of $^{14}CO_2$ were expired from both types of labelled cypermethrin. The elimination of radioactivity within 7 days was 29-33% (^{14}C-benzyl label, male, femal) and 41-56% (^{14}C-cyclopropyl lable, male, female) in the urine and 55-59% and 34-46%, respectively, in the faeces.

Radioactivity derived from the benzyl-^{14}C and cyclopropyl-^{14}C labeling was rapidly eliminated, mostly in the urine following a single oral dose of ^{14}C-cypermethrin, 1.2-2.2 mg/Kg bw, in male and female rats and sacrificed 3 days after dosing ([redacted]). The radioactivity derived from the cyclopropyl-^{14}C labeling was eliminated mainly inthe urine of both males and females (^{14}C-cyclopropyl-label: 66.5 and 55.8%), the remainder was eliminated in the faeces (^{14}C-cyclopropyl-label: 27.0 and 28.7%). Less than 0.1% of the dose was eliminated as $^{14}CO_2$.

In the mouse, radioactivity was rapidly and almost completely eliminated in the urine and faeces following single oral doses of cis- or trans-cypermethrin with either ^{14}C-benzyl or ^{14}C-cyclopropyl labelling ([redacted]). The urine was the major route of elimination for trans-cypermethrin metabolites (66-80%), whereas ot was less important for the elimination of cis-cypermetrhin (31-41%) compared with the faeces.

In addition, there is published human data available. Human dose-excretion studies were performd with cypermethrin (*cis:trans*/1:1) by Eadsforth and Baldwin (1983). Four male volunteers received an oral dose of cypermethrin (2 subjects received a single dose of 0.25 mg and 0.5 mg, respectively; 2 subjects received 2 oral doses of 1.0 mg and 1.5 mg, and 0.25 and 1.0 mg, respectively, separated by a period of 12 months). Twenty-four hour urine samples were collected up to a period of 5 days. After a single oral dose, urinary excretion was rapid and confined to the first 24 h sample: 78% of the *trans* isomer and 49% of the *cis* isomer as free and conjugated cyclopropane carboxylic acid. This confirms that, as in other mammals, ester cleavage and elimination of the *cis* and *trans* cyclopropanecarboxylic acid moieties in the free and conjugated form is a major route of metabolism of cypermethrin in man.

3.1.4 Metabolism

Extensive metabolism studies with cypermethrin have been conducted in a number of species. These studies were peer reviewed by the International Programme on Chemical Safety (IPCS) and the conclusions of this expert group published in Environmental Health Criteria no.82 – Cypermethrin (WHO, 1989).

The overall metabolism (see fig. 3.1.2.) of cypermethrin was found to be qualitatively similar in each of the species studied, based on existing in-vivo studies in rats, mice, dogs and cows. Differences related only to the rate of metabolism rather than the nature of the metabolites formed. The only major species differences related to the type of conjugation reactions which take place prior to elimination.

Hydrolytic cleavage of the ester bond and elimination of the *cis* and *trans* cyclopropanecarboxylic acid and 3-phenoxybenzyl moieties in the free and conjugated form is known to be a major route of metabolism in mammals, including humans. The **cyclopropane carboxylic acid moiety** is mainly and rapidly excreted as the glucuronide conjugate, with only limited hydroxylation of the methyl groups attached to the cyclopropane ring. The cyanide moiety is metabolised to thiocyanate. The **3-phenoxybenzyl moiety** is mainly converted to 3-phenoxybenzoic acid and further conjugated and excreted as the glutamic acid conjugate in the cow, as a taurine conjugate in the mouse and as a glycine conjugate in the rat and dog. Phenoxybenzoic acid is further metabolised to a hydroxyl derivative (3-(4'-hydroxyphenoxy)benzoic acid) and conjugated with glucuronic acid or sulphate. The major route of excretion of metabolites is via the urine.

In **rats** given a single toxic oral dose (200 mg/Kg) of radiolabelled cypermethrin, the route of biotransformation of cypermethrin was equivalent to those described for the repeated administration of sub-lethal doses of cypermethrin (2 mg/Kg). The absorbed *cis*- and *trans*- isomers of cypermethrin were rapidly metabolised via cleavage of the ester bond to yield the *cis*- and *trans*- cyclopropane carboxylic acids which were then excreted mainly as glucuronide conjugates in the urine. The 3-phenoxybenzyl moiety was mainly converted to the 3-phenoxybenzoic acid, most likely via α-hydroxy-phenoxybenzonitrile, with sulphation being the main route of conjugation. There was no evidence for accumulation of unknown metabolites following repeated exposure and no indication of sex or dose dependent changes (Rhodes et. al., 1984).

The identification of cypermethrin metabolites in **mice** has been carried out using radiolabelled forms of the separate *cis*- and *trans*- isomers. Radioactivity from the *trans*-isomer was mainly excreted in the urine and that from the *cis*-isomer in the faeces. As reported for other species, metabolism occurred mainly via ester cleavage and elimination of the *cis*- and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylic acid moieties as the glucuronide conjugates. The α-cyano-3-phenoxybenzyl alcohol released as a results of ester cleavage was mainly converted to 3-phenoxybenzoic acid which was partly eliminated unchanged, partly conjugated with amino acids

(mainly taurine) and glucuronic acid and partly oxidised to 3-(4-hydroxyphenoxy) benzoic acid which was then excreted as the sulphate conjugate (Hutson et al, 1981).

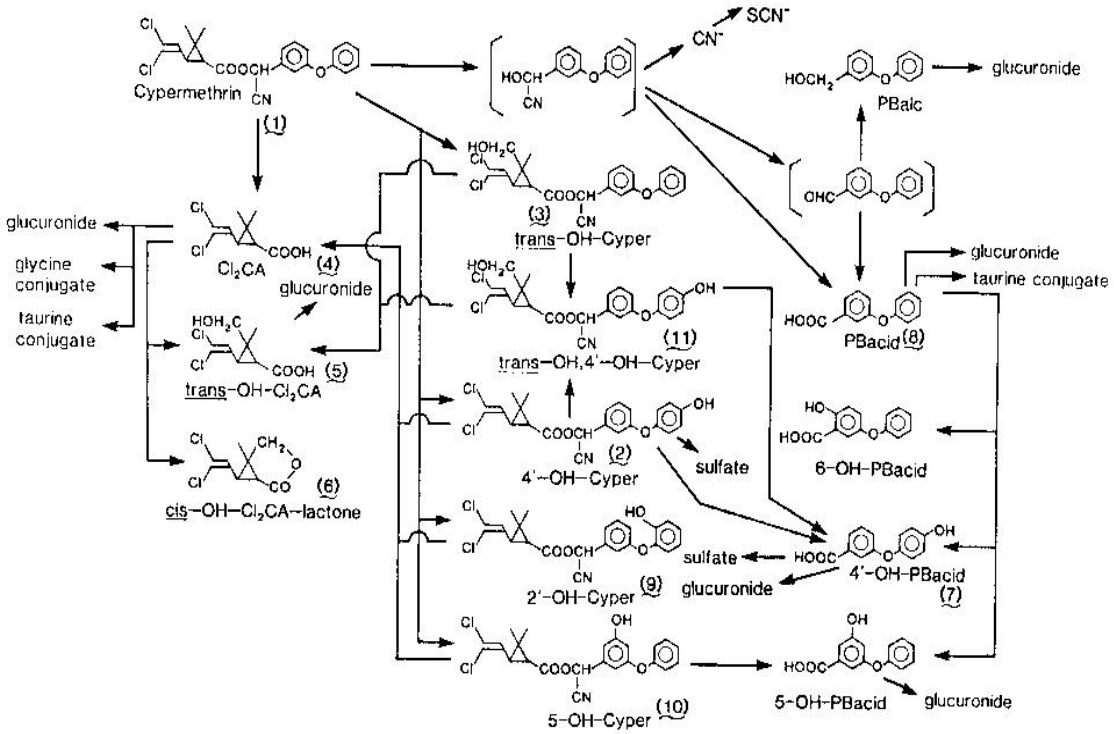
In vitro studies using liver microsomal preparations from rats and mice showed that ester cleavage is more extensive for *trans*- than for *cis*-cypermethrin, while the relative extent of oxidative metabolism of the 2 isomers depends on the enzyme source. The low toxicity of *trans*-cypermethrin to mice to the corresponding *cis*-isomers is consistent with their greater ease of biodegradation in the mouse microsomal system (Shono et al., 1979).

In **man**, the hydrolytic ester cleavage of the cypermethrin molecule and subsequent elimination of the cyclopropyl acid moieties in the free and glucuronidated form is also found to be the major route of metabolism. In a human dose excretion study, four male subjects were given a single oral dose of a 1:1 *cis:trans* mixture of Cypermethrin (0.25 mg to 1.5 mg). Urinary excretion of the free and conjugated 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid was rapid, occurring within the first 24 hours. Subjects excreted 78% of the *trans*- isomer dose and 49% of the *cis*-isomer dose in the form of metabolites (Eadsforth and Baldwin, 1983).

Recently, two human liver microsome pyrethroid-hydrolysing carboxylesterases (hCE-1 and hCE-2) have been identified (Nishi et al., 2006). hCE-1 and hCE-2 hydrolysed the *trans*-isomers of cypermethrin 14-fold and 29-fold faster, respectively, than the corresponding *cis*-isomers. This is consistent with previous animal studies (Stok et al., 2004). It is suggested that hCE-1 and hCE-2 play major roles in the stereoselective metabolism of cypermethrin with a preference for the *trans*-isomers.

A metabolic pathway for cypermethrin in mammals has been proposed by IPCS (see fig.3.1.2).

Fig. 3.1.2. Proposed metabolic pathway for cypermethrin in mammals (WHO, 1982)



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

DERMAL

3.1.5 Percutaneous absorption

In vitro

The percutaneous absorption of [^{14}C]-Cypermethrin *cis:trans*/40:60 through viable human epidermal membranes was studied using a static test system according to OECD guideline 428 by Hardwick (2006). Skin absorption was investigated at two application rates. The high dose rate, nominally 1.0 mg/cm^2, represented the undiluted EC formulation; the low dose, nominally 0.00025 mg/cm^2, represented the diluted spray solution used in normal agricultural spraying operations. The membranes were not occluded and the skin was exposed to the test substance for 8 h, after which time the skin was washed. Due to the low solubility of Cypermethrin, the receptor fluid selected was ethanol/water (1:1, v/v). Receptor fluid was collected up to 24 h post dose. At 24 h post dose the skin was tape stripped 5 times to remove the stratum corneum. Radioactivity was determined in the receptor fluid, residual skin, skin washings, tape strips and diffusion cell washings to determine the overall mass balance of radioactivity. Recovery of radioactivity was essentially quantitative for both dose levels.

Following a 1 mg/cm² application of [^{14}C]-Cypermethrin cis:trans 40:60 (concentrate, 100 g/L), there was a mean lag phase of ca 2.1 hours. The mean maximum rate of absorption was 1553 ng/cm²/h. The mean concentration/time curve showed that absorption slowed down 10 hours following dose application with a mean permeability coefficient (Kp) of 1.6^{-5} cm/h. Absorbed radioactivity, in the receptor fluid, accounted for 1.5% of the applied dose by the terminal timepoint, corresponding to a mean of 21990 ng equivalents/cm^2. The majority of the radioactivity was removed from the skin during the washing procedure at 8 hours following dose application (43.2%). The remainder of the radioactivity was recovered the tape strips (10.5%) or following solubilisation of the residual skin (25.5%). Residual radioactivity extracted from the diffusion chamber accounted for 17.4% of the applied radioactivity.

Following a 0.00025 mg/cm^2 application of [^{14}C]-Cypermethrin cis:trans 40:60 (spray dilution, 25mg/L), there was a mean lag phase of ca 0.16 hours. The mean maximum rate of absorption was 3.328 ng/cm^2/h. The mean concentration/time curve showed that absorption was steady throughout the study and did not plateau. The mean Kp for [^{14}C]-Cypermethrin cis:trans 40:60 at this concentration was 1.3^{-4} cm/h.

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

Absorption of radioactivity was rapid, with detectable levels in the receptor fluid at 1 h. At study termination, 1.5 and 13% of the applied dose were recovered in the receptor fluid for the concentrate (100 g/L) and spray dilution (25 mg/L), respectively. The amount absorbed (found in the receptor fluid) increased only ca 500 fold for a 4,000 fold increase in concentration, i.e. absorption was not proportional to increasing concentration thus indicating saturation of absorption at the higher dose level. The washing procedure removed variable amounts of radioactivity. For the concentrate, the majority of applied radioactivity was removed but, for the spray dilution, the majority remained associated with the skin. The washing procedure had no noticeable effect on the rate of absorption of radioactivity. Approximately 10% of the applied radioactivity was associated with the stratum corneum that was removed during the tape stripping process.

In conclusion, the residual amounts within the skin have to be included. It cannot be excluded that the amount present in the skin could not be potentially absorbed. Dermal absorption values including residual skin are:

Concentrate **27%**, Spray dilution **68.6%.** According to the general consensus at TM (2008) and Mota, the material found in the stratum corneum should also be included in the absorbed dose unless tape stripping data is available that allows to discount the top 25% of the stratum corneum. As there is no information for the tape strips individually, dermal absorption values including residual skin and all 5 tape strips are: Concentrate **37.5%,** Spray dilution: **78.6%.**

Note: this study has not been reevaluated according to EFSA Guidance on Dermal Absorption (2012). At the WG meeting, it was agreed that re-evaluation of this is not essential, as the results from the in vivo study were used for risk assessment.

In vivo

The applicant submitted a new *in vivo* rat absorption study in September 2010 to the RMS, together with a robust summary. The Applicant considers the study as valid and therefore is of the opinion that any human exposure assessment must be revised taking into account the dermal penetration rate obtained in this study. This new data was accepted, evaluated and taken into account by the RMS.

The dermal absorption after single dermal administration of an 500 g/L EC formulation of [^{14}C]-Cypermethrin *cis:trans*/40:60 was studied in the rat according to the OECD guideline 427 by [redacted]. Cypermethrin was tested at two target concentrations: the emulsifiable concentrate (500 g a.i./L) and a representative field dilution (25 mg a.i./L). The study objectives were to determine the extent of percutaneous absorption of the compound related radioactivity, its permeation through the skin into the body, and its elimination *via* excreta following a contact time of 8 hours. The bioavailability of the residues remaining in/on skin after washing of the application site and the kinetics of percutaneous absorption were estimated at 3 time points, 24, 72 and 216 hours after the beginning of the 8 hour exposure time. Furthermore, the distribution of radioactivity between the upper skin layer (*stratum corneum*) and the rest of the skin was estimated. At sacrifice the following samples were collected: 'O'-ring + protective device; skin wash; surface tape strips (stratum corneum), 20 in total, individually sampled; application site (tape stripped); skin (non treated area); whole blood; plasma; gastrointestinal tract; residual carcass. The cage washings at the end of the collection period were also retained.

Total absorption was assessed from recoveries of radioactivity in urine and faecal samples, cage wash, tissues, GI tract and residual carcass as 0.98% of the high dose absorbed within 24 h. The total absorption increased to 1.09% at 72 h and to 3.44% at 216 h after dosing. For the low dose 5.62% of the dose was absorbed within 24 h. The total absorption increased to 8.63% at 72 h and to 11.03% at 216 h after dosing. The systemic dose per day showed a low systemic dose (ca 1%) in the time groups of the high dose and a steady decrease from 5.6% after 24 h to ca 1% after 216 h in the low dose. The highest amount of radioactivity, 0.59% of the administered dose, was the residual amount found in the low dose carcases, at the 24 h sacrifice. Levels in blood and plasma were close to or below the limit of detection. The low dose values were considerably less than 1 ng/g tissue.

Skin strips: In the high dose mean radioactivity in skin strips was 7.11% after 24 h, 6.73% after 72 hours and 5.03% at 216 h after dosing.

For the low dose, residual radioactivity in skin strips was 7.41% after 24 h, 6.54% after 72 hours and 4.34% after 216 h.

These values include all 20 tape strip amounts. Due to the persistence of absorption over the extended observation period and in accordance with current guidance on use of tape strip data (eg EFSA or MOTA 3), the total amount of absorbed material or biologically available material for absorption was adjusted to exclude the amounts recovered at each time point in the first two tape strips – see discussion below.

At both dose levels skin stripping revealed that most radioactivity in the stratum corneum was concentrated in the upper layer and/or hair at 216 h after the start of application and therefore the potential for further absorption was limited.

Stripped skin: Radioactivity in the dermis (stripped skin) increased from 0.82% at 24 h to 1.23% at 216 h after application in the high dose group. For the low dose the radioactivity decreased from 1.12% at 24 h to 0.20% at 216 h after application.

In the high dose group sacrificed 16 hours after the 8 hour exposure, 0.82% of administered dose was found in the skin (after washing the dislodgeable amount and removing the stratum corneum by tape stripping). This residual amount in the skin increased slightly to 1.23% of administered dose when assessed at 216 hours.

For the low dose groups, radioactivity in stripped skin was 1.12% of administered dose after 24 h and decreased to just 0.20% by 216 hours.

Elimination: The majority of the radioactivity associated with administered Cypermethrin was removed by washing. The dislodgeable, non –absorbed radioactivity in the skin wash was 84-91% of the applied radioactivity for the low dose and in the high dose circa 91% of the applied radioactivity was removed in the skin wash.

Additionally significant amounts of radioactivity were associated with the ‘O’-ring and cover - 6-8% in the high dose group and slightly lower, 2.0-2.5%, in the low dose.

Urinary excretion accounted for 2.26% and 8.18% of the high and low dose, respectively, measured over the 216 h assessment period.

Urinary excretion at 24 and 72 hours equated to 0.28% and 0.71% of the administered dose in the high dose group and 2.74% and 6.43% in the low dose.

Faecal excretion accounted for small amounts of radioactivity - 0.38% and 2.08% of the administered high and low doses respectively, within 216 h. High dose faecal excretion at 24 or 72h was 0.07% and 0.15%. The values for the low dose group were 0.38% and 1.12% respectively.

Based on the results of this study and taking account of the Manual of Technical Agreements (MOTA3) Biocides Technical Meeting, 24 Feb, 2010, the RMS considered *in vivo* percutaneous absorption study of an EC formulation in rats (concentrate 500g ai/L; spray dilution 25 mg a.i./L = agriculture field dilution, 8 h exposure, post exposure times 24h, 72h, 216h), revealed dermal absorption values, including residual skin (stripped skin) at 24h, 72h or 216 h after dosing:

Concentrate: 1.8, 2.1, 4.7%

Spray dilution: 6.5, 9.2, 11.2%.

The dermal absorption values including residual skin and **all 20** tapestrips at 24h, 72h or 216 h after dosing:

Concentrate: 8.9, 8.9, 9.7%

Spray dilution: 13.94, 15.8, 15.6%.

The draft 'Guidance on Dermal Absorption' recently circulated by EFSA Panel on Plant Protection Products and their Residues (PPR) seeks to clarify the use of data obtained by skin stripping. In the current study, a total of 20 strips were used and the total dermal absorption values calculated above reflect either the inclusion of results from all strips or from none. Neither position is thought to accurately reflect the role of the stratum corneum as a partial barrier to dermal absorption and a means of removal of non-absorbable material. The EFSA Guidance reflects the conclusions of MOTA3 in the Biocides Technical Meeting. Where there is no evidence for continuing absorption at the end of the observation period, it is justified to exclude the tape strip data entirely. In cases where more than 75% of absorption occurs within half of the observation period, then tape strip data may also be excluded unless only pooled data are available. In this study the absorption after 72 hours was less than 75% of the total after 216 h and so inclusion of some tape strip data is considered appropriate. There is general agreement, reflected in both MOTA3 (4.1.1 Q2) and the most recent EFSA Guidance on Dermal Absorption (point 5.1.1) that the amount of dose removed by the initial two tape strips represents material that will not become biologically available and that practically and pragmatically these data can be excluded for the total absorption calculation.

Based on these assumptions the dermal absorption values for cypermethrin including total absorbed, residual skin absorption and 18 tape strips (first two excluded) at 24 h, 72 h or 216 h after dosing are:

Concentrate:	**6.7,**	**7.0,**	**7.6%**
Spray dilution:	**12.5,**	**13.6,**	**12.7%**

Human data from the open literature

Cypermethrin was administered dermally on the dorsum to 6 male volunteers at a dose of 31 mg/800 cm² (*cis:trans*/56:44, purity 91.4%) as a soya oil-based formulation, 26 mg a;s./ml (Woollen et al., 1992). The area remained unoccluded for 8 hours and was then washed with 1ml of 3% aq. Teepol. The volunteers then wore a T-shirt until taking a shower 24 hours after dosing. Urine was recovered over the periods 0-4, 4-8, 8-12 h post treatment and then over 12h intervals up to 120 hours after dosing. Total volume, creatinine concentration and pH were analysed in the samples together with linear regression analysis of half-life – assessed on excretion rate versus mid-point time from 18 h post treatment t the point at which metabolites fell below the limit of detection. Urinary metabolites of Cypermethrin (DCVA, 3PBA, 4OH4PBA and 2OH3PBA) were analysed together with internal standards 4PBA and 4OH4PBA. The rat metabolite, 2OH3PBA, was not detected in human samples. The limit of detection was typically 0.5µg/L for all four metabolites and the precision is cited in the publication to be typically 5% relative standard deviation.

41% of the dermal dose (range 36-48%) was recovered in the detergent skin wash after the 8h exposure period. A further 24% was recovered from the T-shirt cover worn overnight. On average, in this study at least 65% of the applied dose was not absorbed.

Cypermethrin metabolites were detectable in the majority of urine samples over the first four hours of exposure but peak excretion rates occurred between 12 and 36 hours post dosing. No metabolites were detected beyond the 96 h sampling point (except for trace amounts of 4OH3PBA in two individuals). The elimination half life for total metabolites was 13 h (range 8-22h, standard deviation ±5.1 h). The average *trans:cis* DCVA ratio was 1:1.2. The amounts of cyclopropane acid metabolites in urine samples following dermal application were circa four times lower than the metabolites derived from the phenoxybenzyl moiety. This indicated that the cyclopropane acid part of the molecule may either not be

effectively absorbed, or more probably is converted to other metabolites which are not measured using the assay procedure. The estimate of cypermethrin dermal absorption, based on *cis* or *trans* DCVA metabolite presence, was 0.3%, this was much lower than the same estimate based on 3PBA and 4OH3PBA – mean of 1.2% dermal absorption estimated. A recovery of only 66.6%was calculated.

It has to be noted that there is no mass balance reported. The purpose of this study was to provide a basis for interpretation of urinary metabolite data in studies of worker exposure, not a complete accounting of administered dose.

Conclusion on dermal absorption

Dermal absorption was studied in the rat, using human tissue, and in human volunteers.

No studies are available for the pure active substance. Studies *in vivo* and *in vitro* were performed using typical formulations, concentrated or as spray dilution, and an 8 hour exposure.

Based on the results of the *in vitro* dermal absorption in human skin study performed with an EC formulation, the dermal absorption values for cypermethrin including total absorbed, residual skin absorption and 5 tape strips (all tape strips) at 24 h after dosing are for the concentrate (100 mg a.s./L) 37.5%, and for the spray dilution (25 mg a.s./L) 78.6%.

Based on the results of the *in vivo* rat dermal absorption study performed with an EC formulation, the dermal absorption values for cypermethrin including total absorbed, residual skin absorption and 18 tape strips (first two excluded) at 24 h, 72 h or 216 h after dosing are for the concentrate (500 mg a.s./L):6.7%, 7.0%, 7.6%; and for the spray dilution (25 mg a.s./L): 12.5%, 13.6%, 12.7%. The total absorption increased over time. This was expected as pyrethroids are stored in the skin following dermal exposure and are slowly released in to the systemic circulation.

The outcome of the rat study is supported by the human volunteer study by Woollen et al., 1992. The estimated dermal absorption based on the phenoxybenzoic acid metabolites 120 h after dosing is 1.2% (range 0.85 to 1.8%) for a 26g/L formulation (applied dose 31mg/800cm²), and only a recovery of 66.6% (skin wash, T-shirt, urine metabolites).

It is well known that the rat skin is more permeable than human skin and it is also well known that the *in vitro* findings generally overpredict the *in vivo* situation ([redacted], Toxicol. Appl. Pharmacol. 243, 239-259). Moreover, the *in vitro* system used was a static test system, whereas today the more reliable flow-through systems are generally used. In the human volunteer study a recovery of only 66.6%was calculated. However, the purpose of this study was to provide a basis for interpretation of urinary metabolite data in studies of worker exposure, not a complete accounting of administered dose. No mass balance was reported.

Therefore, the recently performed *in vivo* dermal absorption study in rats ([redacted]) provides the most reliable dermal absorption data.

For the assessment of the human internal dermal exposure to the formulation, a value of **13%** is used.

INHALATION

Pyrethroïds are rapidly absorbed in humans following inhalation exposure, but no estimates are available regarding how much of an inhaled dose is absorbed for cypermethrin. Consequently, in the risk characterisation a value of 100% absorption is used following inhalation exposure.

EXPOSURE TO THE DEVELOPING FOETUS / EXPOSURE VIA BREAST MILK

The placenta does not represent a very effective barrier. Limited information is available regarding the ability of pyrethroïd compounds to cross the placenta and be distributed to the fetus. In the teratogenicity studies in rats and rabbits, no teratogenic effects were observed. Nevertheless, other rat studies do indicate in utero exposure to cypermethrin and that this may result in persistent effects on neurotransmitters and on the immune system ([redacted]), see DocIIA, section 3.8.1 Teratogenicity.

Exposure via the breast milk is possible. Lipid –soluble xenobiotics diffuse along with fats from plasma into the mammary gland and are excreted with milk during lactation. Most probably, excretion into breast milk occurs via lipid diffusion across membranes with retention in milk fat.
Evidence of infant exposure via breastfeeding has been published in the open literature:
Bouwman et al., 2006. Simultaneous presence of DDT and pyrethroid residues in human breast milk from a malaria endemic area in South Africa. Environmental Pollution, 144: 902-917.
[redacted]. Comparing water, bovine milk, and indoor residual spraying as possible sources of DDT and pyrethroid residues in breast milk. J. Toxicol. Environ. Health A, 72: 842-851.

Based on these considerations, the F0-, F1-and F2-generations were sufficiently exposed to cypermethrin and its metabolites during foetal development and lactation. The exposure did not lead to any effects on reproduction in the 3-generation study.

3.1.6 Conclusion

The absorption, elimination, and distribution of cypermethrin (cis:trans/40:60) was studied in well performed single and repeated dose studies with ^{14}C-cyclopropyl or with ^{14}C-phenyl radiolabelled cypermethrin doses (single: 3 and 50 mg/Kg bw, repeated 3 mg/Kg bw) by oral gavage in the rat ([redacted]). The findings are in agreement with data from the older published literature.

Absorption of cypermethrin from the gastro-intestinal tract of the rat is rapid but incomplete. Urinary and faecal excretion was 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. At the low dose (3 mg/Kg bw) 51.3% to 52.8% of the dose was absorbed by the male rats and 43.6% to 57.6% in case of the females.At the high dose level (50 mg/Kg bw), 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. For the estimation of oral absorption, a conservative approach is adopted. Different values were adopted for animals and humans, based on the low dose (3 mg/Kg bw) data of the [redacted]. For **animals**, an oral absorption value of **44%** is adopted for deriving systemic NOAELs (PODs for the AELs are closer to the low dose rather than the high dose). For the estimation of **human** systemic exposure, an oral absorption value of **57%** is adopted.

Distribution. Following repeated daily oral dosing of 3 mg [^{14}C-phenyl]-cypermethrin, the levels of radioactivity in inguinal and peri-renal fat 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 (for both males and females), and the levels in the fat falling by 2-7 times: in males in peri-renal fat (2-fold), and in females in brown fat (7-fold).

Excretion. The excretion was rapid, being virtually complete by 72 h following a single oral dose of [^{14}C-cyclopropyl]- or [^{14}C-phenyl]-cypermethrin at a dose of 3 or 50 mg/Kg bw. Urinary and faecal excretion was similar at the low dose for both radiolabels, but at the higher dose level faecal excretion predominated, especially in the males.

Metabolism. Hydrolytic cleavage of the ester bond and elimination of the *cis*- and *trans*-cyclopropanecarboxylic acid and 3-phenoxybenzyl moieties in the free and conjugated form is known to be a major route of metabolism in mammals, including humans. The cyclopropane carboxylic acid moiety is mainly and rapidly excreted as the glucuronide conjugate, with only limited hydroxylation of the methyl groups attached to the cyclopropane ring. The 3-phenoxybenzyl moiety is mainly converted to 3-phenoxybenzoic acid which is further metabolised to a hydroxyl derivative (3-(4'-hydroxyphenoxy)benzoic acid) and conjugated with glucuronic acid or sulphate. The major route of excretion of metabolites is via the urine. In faeces, most of the radioactivity is unchanged compound. The metabolism of cypermethrin is stereoselective with a preference for the *trans*-isomers (human and animal data).

Dermal absorption. The *in vivo* dermal absorption study in rats provided the most reliable dermal absorption data. The dermal absorption of cypermethrin determined in rats *in vi*vo resulted in an absorption of 7.6% and 12.7% of the applied dose for the concentrate (500 g/L) and spray dilution (25 mg/L). For the assessement of the human internal dermal exposure, a value of **13%** is used.

Absorption by inhalation. Pyrethroïds are rapidly absorbed in humans following inhalation exposure, but no estimates are available regarding how much of an inhaled dose is absorbed for cypermethrin. Consequently, in the risk characterisation a value of **100%** absorption is used following inhalation exposure.

3.2 ACUTE TOXICITY

The most relevant studies are fully described. The summaries of the other studies are available as IUCLID entries and summarised in table 3.2. In most of these studies necropsy findings were generally missing.

Table 3.2.: Acute toxicity of cypermethrin

Route	Method Guideline	Test substance	Species Strain Sex no/group	Dose levels duration of exposure	Value LD50/LC50	Reference
Oral	OECD 423	**Cypermethrin *cis:trans*/40:60** Purity 94% CMN92T1197A N	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]
Oral	Similar to OECD 401	**Cypermethrin *cis:trans*/40:60** 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]

Oral		**Cypermethrin *cis:trans*/37:63** 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)**	[redacted]
Oral	Litchfield and Wilcoxon, 1949	**Cypermethrin** purity 93.5%	Rat: CFY strain 60 males, 60 females	Dose levels: not mentioned, gavage, vehicle: olive oil	**250 mg/Kg bw (m,f)**	[redacted]
Oral	Similar to OECD 401	**Cypermethrin *cis:trans*/50:50** WL43467	Rat : Wistar male/female 6/sex/group	100, 157, 253, 404, 639, 1016 mg/Kg bw (**10%** solution **in corn oil**) 197, 313, 496, 791, 1258, 2000 mg/Kg bw (20% solution in corn oil)	**287 mg/Kg bw** (10% solution, m+f) 371 mg/Kg bw (20% solution, m+f)	[redacted]
Oral	Similar to OECD 401	**Cypermethrin *cis:trans*/40:60** CGA 55186 tech	Rat: Tif:RAIf (SPF) male/female 5/sex/group	5000 mg/Kg bw, aquous suspension in **CMC**; 14 days post exposure period	>5000 mg/Kg bw (m,f)	[redacted]
Oral	Similar to OECD 401	**Cypermethrin *cis:trans*/40:60** CGA 55186 tech	Rat: Tif:RAIf (SPF) male/female 5/sex/group	2000, 3500, 5000, 6000 mg/Kg bw in **PEG 400**; 14 days post exposure period	3863 mg/Kg bw (m,f)	[redacted]
Oral	Similar to OECD 401	**Cypermethrin *cis:trans*/40:60** CGA 55186 tech	Mouse: Tif:MAG (SPF) male/female 5/sex/group	1500, 3000, 4000 mg/Kg bw in **PEG 400**; 14 days post exposure period	2011 mg/Kg bw (m,f)	[redacted]
Oral	Similar to OECD 401	**Cypermethrin *cis:trans*/40:60** CGA 55186 tech	Rabbit: New Zealand White male/female 3/sex/group	0, 600, 1000, 6000 mg/Kg bw in **PEG 400**; 14 days post exposure period	>6000 mg/Kg bw (m,f)	[redacted]
Dermal	Similar to OECD 402	**Cypermethrin *cis:trans*/40:60** CGA 55186 tech	Rat: Tif:RAIf (SPF) male/female 5/sex/group	Limit test: 2000 mg/Kg bw, Application on the back under occlusive conditions for 24 h	**>2000 mg/Kg bw (m,f)**	[redacted]
Dermal	Guinea-pig flank model	**Cypermethrin *cis:trans*/55:45** (purity>90%)	Guinea-pig, albino	100µl at 0.001, 0.01, 0.1, 1.0, 10% applied to right flank (clipped free) – 30 mm². Observation 0.5, 1, 3, 6 h after application	Not determined. Cutaneous effects: attempted licking and biting at application site	[redacted]
Inhalation	OECD 403	**Cypermethrin *cis:trans*/40:60**	Rat: Tif:RAIf (SPF) male/female	0, 970, 1926, 3462, 5328 mg/m³; in ethanol;	**3281 mg/m³ (m)** 5038 mg/m³ (f)	[redacted]

		CGA 55186 tech	5/sex/group	aerosol, 4h, nose only; 14 days post exposure period	3894 mg/m³ (m,f)	

Summary of acute toxicity: (from Doc.III-A 6.1.1, 6.1.2, and 6.1.3)

Oral

Cypermethrin 40:60. Moderate oral toxicity, tested in the rat (LD_{50} = 1732 mg/Kg bw; [redacted]). Mortality was observed 1 day after dosing, starting from 1200 mg/Kg bw on. Dyspnea, exophthalmus, ruffled fur and curved body position was observed in all test groups. Diarrhea 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. 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.

Cypermethrin 40:60. Moderate oral toxicity, tested in the rat (LD_{50} = 500 mg/Kg bw; [redacted]). The LD_{50} cut-off value was determined with the acute toxic class method. 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 LD_{50} was found to be 500 mg/Kg.

Additional information (no key studies) was found in other reports and in the open literature:

Cypermethrin 50:50. Moderate oral toxicity, tested in the rat (LD_{50} = 287 mg/Kg bw; [redacted] in PPP DAR cypermethrin). This study was discussed in depth by BE in the PPP DAR in the scope of Directive 91/414/EEC. All deaths occurred with 3 days (10% solution in corn oil) or 4 days (20% solution in corn oil). Clinical symptoms recorded were dyspnea, lethargy, clonic convulsions, piloerection, abasia, tremor, salivation, splayed hind leg gait, tip-toe walk, blood around nose and mouth, abnormal respiratory noise. All survivors had regained any initial bw loss by the end of the 14 days post exposure period.

[redacted] reported in a short communication 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. The oral LD_{50} = 250 mg/Kg bw was also confirmed in [redacted].

In conclusion: The oral toxicity of cypermethrin varies with the type of vehicle used and the isomer ratio. In general, aqueous suspensions were the least toxic and non-polar solutions the most toxic. The acute toxicity of the racemic mixture is also determined by the isomer ratio, with the *cis*-isomer found to be the most toxic (WHO, 1989). Oral LD_{50} values vary from 250 mg/Kg (in oil) to >5000 mg/Kg (in aqueous solutions). Nevertheless, the toxic responses in all species were found to be qualitatively similar. 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. These signs appear within 1 hour after dosing and survivors recovered within 10-12 days.

Dermal

Cypermethrin 40:60. Low dermal toxicity, only tested in the rat, but with no mortalities, nor local reaction, noted at the limit dose of 2000 mg/Kg bw (LD_{50} > 2000 Kg/Kg bw; [redacted]). Clinical signs were characterised by dyspnea, ruffled fur, curved and ventral body position. In addition, a transient diarrhea was observed. Animals recovered within 10 days.

Data from the open literature: Pyrethroid-induced paraesthesia in the guinea-pig flank model ([redacted]). Dermally applied cypermethrin (1%) elicited a behavioural response in the guinea-pig flank model characterised by attempted licking and biting at the application site. Licking behaviour was evident 30 min following application and peaked at 1 h. No further increase in activity was observed when cypermethrin 10% was applied, although the duration of the behavioural response was prolonged.

Inhalation

Cypermethrin 40:60. Moderate acute inhalative toxicity, only tested in the rat. An LC_{50} = 3281 mg/m^3 (aerosol) was determined by nose-only exposure for 4 hours ([redacted]). Animals died during or within 2 hours following exposure. 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. 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. Body weight was not affected in females. At necropsy, 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/m^3, dilatations of the heart were found.

For classification and labelling of cypermethrin, we base our conclusions only on the acute oral, dermal, and inhalation toxicity data obtained from studies performed with cypermethrin *cis:trans*/40:60. In conclusion, cypermethrin is of moderate acute oral and inhalation toxicity, but of low dermal toxicity.

Classification/Labelling of the active substance 'cypermethrin *cis:trans*/40:60' for acute toxicity according to the criteria in Directive 67/548/EEC:

Xn, R22, R20: **Harmful if swallowed**. **Harmful by inhalation.**

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, H332: **Harmful if swallowed**. **Harmful if inhaled.**

3.3 IRRITATION AND CORROSIVITY

Table 3.3.1: Skin irritation of cypermethrin

Species	Method	TS	Average score 24, 48, 72 h (EU methodology)		Reversibility yes/no	Result	Reference
			Erythema	Edema			
Rabbit	OECD 404	cypermethrin 40:60 CGA 55186 tech	1.33/1.33/2.0	0.33/0.33/0.67	Yes, recovered after 7 days	**Not classified**	[redacted]

Table 3.3.2: Eye irritation of cypermethrin

Species	Method	TS	Average score 24, 48, 72 h (EU methodology)				Reversibility yes/no	Result	Reference
			Cornea	Iris	Conjunctiva Redness	Conjunctiva Chemosis			
Rabbit	OECD 405	Cyp. 40:60 CGA 55186 tech	0/0/0	0/0/0	2/1.33/1.67	1/0.33/0.67	Redness reversible after 7 days Chemosis reversible after 72 hours	**Not classified**	[redacted]

Table 3.3.3: Relevant human data (see 3.10 for a detailed discussion) and respiratory tract irritation data

Species	Route of exposure	TS	Observations	Result	Reference
Human: case report	inhalation	Cypermethrin 0.25%	5 cases of respiratory irritation after exposure through the air-conditioning system in an office. Clinical signs: itching of eyes, nausea, sore throat, shortness of breath. 7 months post-exposure: still 3 patients with significant pulmonary dysfunction, complaining of cough, congestion, and wheezing.	Irritating	Lessenger (1992)
Human: health survey	inhalation, dermal	Pyrethroids, including 10% cypermethrin emulsion	Health survey: 199 workers engaged in dividing and packaging pyrethroids (deltamethrin, fenvalerate, cypermethrin). Sneezing and increased nasal secretion.	Irritating	He et al., 1988

Skin and eye irritation

Cypermethrin *cis:trans*/40:60 is found to be slightly irritant to rabbit skin ([redacted]), but does not require classification.

Cypermethrin *cis:trans*/40:60 is also slightly irritant to the eyes in the rabbit ([redacted]), but does not require classification.

Respiratory tract irritation

Acute toxicity and repeated dose toxicity studies performed with rats revealed that cypermethrin has a respiratory irritation potential.

Respiratory tract irritation caused by cypermethrin is characterised by cough, mild dyspnoea, sneezing, and rhinorrhea.

Lessenger (1992) reported 5 cases of poisoning by aerial exposure to cypermethrin. After treatment of the air-conditioning system with cypermethrin, creating a fine vapour, employees were allowed to enter the treated building after 2 days. Already after 5 minutes the exposed employees experienced shortness of breath, dyspnea, wheezing, cough, congestion, nasal discharge, burning eyes, itching skin, nausea, and headaches. The employees could re-enter the building repeatedly and when they did, they experienced a return of their symptoms after turning on the air-conditioning system. Five employees presented for examination. Shortness of breath persisted for over 2 weeks, and sore throat and sinus infections were still persistent 7 months post-exposure in one patient (non-smoker). Three other patients (two smokers) without previous pulmonary problems developed significant respiratory dysfunction (still complaining of cough, congestion, and wheezing) 7 months post-exposure.

Sneezing and increased nasal secretion was a common feature for workers engaged in dividing and packaging pyrethroids as described by He et al. (1988).

According to the observations in animals and findings on exposure of the general public to cypermethrin, it is concluded that cypermethrin must be regarded as a respiratory tract irritant.

Cypermethrin requires classification for respiratory tract irritation.

3.4 SENSITISATION

Table 3.4.1.: Skin sensitisation of cypermethrin

Species	Method	TS	Number of animals sensitized/total number of animals	Result	Reference
CBA/CaCrl Mice	OECD 429 (LLNA)	Cypermethrin 40:60	Induction: Cypermethrin 2.5%, 1%, 0.5% in 80% Acetone in Olive Oil. Stimulation index (SI) < 3.0 0.5% SI=0.35; 1% SI=0.46; 2.5% SI=0.63 Positive control : SI=4.43	Not sensitising	[redacted]
Guinea pig	OECD 406 (M&K)	Cypermethrin (92-97.5 % purity)	Intradermal induction: 5% in corn oil/FCA; percutaneous induction: 100%; challenge: 100%. No erythema or oedema at application site for 48h after challenge patch was removed.	Not sensitising	**Pore (1993)** in 91/414 DAR for cypermethrin made by the BE CA.
Guinea pig	M&K	WL 43467 Cypermethrin (50:50)	2/20: showed moderate erythema following the challenge procedure. Intradermal induction: 0.5% in corn oil/FCA; topical induction: 25% in corn oil; challenge: 20% in corn oil.	Very weak skin sensitising	**Coombs et al., 1976**

A well performed Local Lymph Node Assay gave no indication that cypermethrin *cis:trans*/40:60 is a skin sensitiser ([redacted]).
An older study performed with guinea pigs ([redacted]) was confirmatory, indicating that cypermethrin is no skin sensitiser. This study was discussed in the 91/414 DAR for cypermethrin by the BE CA, and was found acceptable.

However, according to [redacted] results from preliminary experiments performed with technical cypermethrin (50:50) indicate that technical cypermethrin had a weak skin sensitising potential.

In contrast with the test results obtained with Cypermethrin *cis:trans*/40:60 in laboratory animals, skin sensitisation (contact sensitivity and eczema) in humans is occasionally reported (Lisi, 1992). One case report describes the possible exposure to spilt cypermethrin resulting in a general urticarial eruption the following day which progressed to involve the eyelids (Wagner, 1994). In conclusion, there are indications, both animal and human, that ***technical cypermethrin*** may have a mild skin sensitising potential. Apart from these (scarce) findings, ***cypermethrin cis:trans/40:60*** is not found to be a skin sensitizer.

Table 3.4.2.:Relevant human data (see 3.10 for a detailed discussion)

Species	Route of exposure	TS	Observations	Result	Reference
Human: Case report	dermal	Cypermethrin techn.	40-year-old woman exposed after a spill contaminating the floor of a bedroom and closet. Next day: generalized urticarial eruption, progressed involving eyelids.	Sensitising	Wagner (1994)
Human	patch test	Cypermethrin in pet. 5%, 2%, 1%	230 volunteers (agricultural workers) patch tested with pyrethroids. Patch test on upper back, read after 2 and 3 days: in 1 subject all 3 concentrations were positive.	Very slight sensitising	Lisi (1992)

Summary irritation/skin sensitisation: (from Doc. III-A 6.1.4, A 6.1.5, and A 6.12)

Skin irritation: Cypermethrin *cis:trans*/40:60 has no skin irritant properties.

Eye irritation: Cypermethrin *cis:trans*/40:60 has no eye irritant properties.

Respiratory tract irritation: Based on animal data and human findings, cypermethrin *cis:trans*/40:60 is found to be irritant to the respiratory tract.

Skin Sensitisation: Cypermethrin *cis:trans*/40:60 is not a sensitizer.

Respiratory sensitisation : It is a recent endpoint introduced by GHS/CLP regulation. The toxicological effect of these active substance were discussed and approved for the inclusion of cypermethrin as an approuved active substance for PT8. No new data were provided for the PT18 assessment and the Human Health effects were not sufjected to revision. Consequently, there is no data available to draw a conclusion for this endpoint.

Classification/Labelling of the active substance 'cypermethrin *cis:trans*/40:60' for irritation/corrosivity/skin sensitisation according to the criteria in Directive 67/548/EEC:

Xi, R37: **Irritating to respiratory system.**

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

STOT SE3; H335: **May cause respiratory irritation.**

Note: Cypermethrin cis:trans/80:20 is classified with R37/38 and R43 (discussed by the BE RMS in the PPP DAR in the scope of Directive 91/414/EEC). The irritation and sensitisation studies concerning cypermethrin cis:trans/80:20 were not submitted and not requested because data concerning the 80:20 isomer ratio is not required for the submission for Annex I inclusion of cypermethrin cis:trans/40:60 in the scope of Directive 98/8/EC.

3.5 SHORT-TERM REPEATED DOSE, SUBCHRONIC AND CHRONIC TOXICITY

Table 3.5.1.: 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	Cypermethrin 50:50 (WL43467)	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	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 Organ weight: ↑ abs. and rel; liver weight in ♀ Clinical chemistry: ↑ hemoglobin and blood urea conc. in ♂; ↑ plasma alkaline phosphatase in ♀ **Neurotoxicity**	1500 ppm 75 mg/Kg bw/d	750 ppm 37.5 mg/Kg bw/d	[redacted]
Oral feed	Method B7 with deviations: limited inquiries, absence of raw data	Cypermethrin 50:50 (WL43467)	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 convulsed during week 1 and 5. Bw gain, food intake, terminal bw: ↓ bw gain Organ weight: ↑ rel. thyroid weight in ♂&♀ Clinical chemistry: ↑ WBC and KCCT at week 5 in ♂; ↑ blood urea levels, ↓ blood glucose levels at week 5 in ♀ **Neurotoxicity**	1500 ppm 37.5 mg/Kg bw/d	150 ppm 3.75 mg/Kg bw/d	[redacted]

i.p.	No guideline	Cypermethrin 50 :50, 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. Clinical signs: scratching, salivation, somnolence, ataxia, convulsion and hind limb paralysis noted at every time point. Bw: reduced at d7. Organ weight: ↑ rel liver weight (20%) Clinical chemistry: ↓serum albumin 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	[redacted]
Oral	No guideline	Cypermethrin, unknown purity	3 weeks	Rat albino males	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	[redacted]
Oral	No guideline	Cypermethrin, 91%	5 days	Rat, Wistar males	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	Not determined	Not determined	[redacted]
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	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:** ↓ food intake, bw gain, weight of gonads 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] in 91/414 DAR for cypermethrin made by the BE CA

The 5-week short term oral toxicity of cypermethrin was investigated in rats and dogs ([redacted]). 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 reduced in both males and females. 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. NOAEL oral, rat, 5 weeks = 37.5 mg/Kg bw/d. Dogs were the 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. Two animals (1 female, 1 male) convulsed during week 1 and 5 respectively. No mortality occurred. Body weight gain was reduced due to the observed loss of apetite. 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 increased at week 5. Relative thyroid weight was increased in both males and females. NOAEL oral, dog, 5 weeks = 3.75 mg/Kg bw/d.

Apart from the clear neurotoxicity observed in these studies, the open literature also demonstrates hepatotoxicity. Clear hepatotoxicity was found at 300 mg/Kg bw/d (i.p., 7 days) and rat liver ATPase was inhibited after a 3 week oral treatment with 31.5 mg/Kg bw/d. Oxidative stress induced by cypermethrin exposure is shown in cerebral and hepatic tissues in rats by an elevation of the level of thiobarbituric acid reactive substances (TBARS) and conjugated dienes.

Administration of cypermethrin in a 21-day dermal toxicity study 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 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. NOAEL dermal, rabbit, 3 weeks = 20 mg/Kg bw/d (91/414 DAR for cypermethrin).

Table 3.5.2.: 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.	Cypermethrin, WL43467, 98.5%	90 days	Rat, CD m/f 12/sex/group	0, 25, 100, 400, 1600 ppm. 0, 1.25, 5, 20, 80 mg/Kg bw/d. Daily	No mortality occurred in the study at any dose level **25, 100, 400 ppm:** General health and behaviour unaffected. **400 ppm:** ↑ kidney weight in ♂ (5%) **1600 ppm:** Clinical signs: Ataxia, hypersensitivity and abnormal gait during the first 5 weeks. 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%), ↓ food intake (♂, ♀), ↓ Hb (♂ 4%, ♀ 6%), ↑ urea (♂ 20%, ♀ 39%), ↑ kidney weight (♂ 7%, ♀ 14%), Males: ↓KCCT (11%), ↑ K^+ (13%) Females: ↓ RBC (6%), ↑ AP (40%), ↑ liver weight (10%), ↑ spleen weight (17%)	80 mg/Kg bw/d	20 mg/Kg bw/d	[redacted]
Oral feed	Deviating OECD 408: Means: standard deviation not calculated.	Cypermethrin, WL43467, 98.5%	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%). Mortality: 2 ♂ and 2 ♀ were sacrificed during week 6 and 10, 10 and 12, respectively, for humane reasons. Haematology: ♀ ↓ RBC (6%), ↓ KCCT (kaolin-cephalin clotting time) (21%). Pathology: focal bronchopneumonia in several animals. **Neurotoxicity**	37.5 mg/Kg bw/d	12.5 mg/Kg bw/d	[redacted]

Oral	No guideline	Cypermethrin, technical grade	90 days	Rat, albino male	0, 5, 10, 20, 40 mg/Kg bw/d. vehicle: ground nut oil daily	Dose-dependent decrease in delayed type hypersensitivity reaction on d61 post-treatment; **20 mg/Kg bw/d** ↓spleen weight, ↑ adrenal weight **40 mg/Kg bw/d** ↓spleen weight, ↑ adrenal weight, leucopenia on d90 **Immunotoxicity**	20 mg/Kg bw/d	10 mg/Kg bw/d	Varshneya et al. (1992)
Oral	No guideline	Cypermethrin 25% EC	12 weeks	Rabbit New Zealand White Male, n=6	0, 24 mg/Kg bw every other day	↓ 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.	24 mg/Kg bw every other day	< 24 mg/Kg bw every other day	[redacted]

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

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]). MTD was reached. As in the 5 week studies, the target tissue/organ was the nervous system and liver. In the 80 mg/Kg bw/d group rats showed hypersensitivity and abnormal gait during the first 5 weeks of the experiment. Of this group, 1 male died and 3 males were killed. 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 reduced throughout the experiment. An increase in kidney (m/f), liver (f), and spleen (f) weight was observed. Both in males and females, plasma urea was increased and haemoglobin was decreased. 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. The health and behaviour of animals in the dose group up to 20 mg/Kg bw/d were unaffected by ingestion of cypermethrin.

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]). The feeding of 37.5 mg/Kg bw/d caused diarrhea, licking and chewing of the paws, whole body tremors, a stiff exagegerated 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. Food intake and body weight gain were reduced throughout the experiment. RBC count and KCCT were decreased in the female dogs. Non-specific pathological changes, mainly in the lung (focal bronchopneumonia) were found in the animals of this group, 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.

This was also confirmed in the more recent subchronic open literature studies (rats, rabbits). These studies reported also that cypermethrin induced a dose-dependent decrease in delayed type hypersensitivity (DTH) reaction in rats and induced moderate toxic effects on hemato-biochemical functions including hematological parameters and profiles of lipid, lipoproteins, protein, urea, creatinine, glucose and total bilirubin of rabbits when administrated orally 24 mg/Kg bw every other day for 12 weeks ([redacted]). In rats, the oral administration of 40 mg/Kg bw/d for 13 weeks resulted in a leucopenic response, substantiating the immunotoxic potential of cypermethrin (Short communication: [redacted]). In this study the NOAEL was 10 mg/Kg bw/d. See section 3.10.1 (Immunotoxicity) for a more comprehensive discussion.

Table 3.5.3.: 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.	Cypeermethrin 50:50, WL43467, 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 ↑ (♀ 4%) Clinical chemistry:liver PNOD ↑ (♂,♀), urea ↑ (♂ 58%), AP ↓ (♂ 33%) Other (minor) changes in hematological and clinical chemical parameterswere not considered of toxicological significance as not supported by histopathological or other evidence of tissue damage. Organ weights: Testes, rel.: ↓ (♂, 6 months) Liver, abs, rel: ↑ (♂, 18 mth) Heart, rel: ↑ (♂, 6 mth) Heart, abs: ↓ (♂, 12 mth) Kidney, rel: ↑ (♂, 12 mth; ♀, 6 mth) Kidney, abs:↑ (♂, 18 mth)	1000 ppm 50 mg/Kg bw/d	100 ppm 5 mg/Kg bw/d	[redacted]

Rodent

Considering the chronic toxicity of cypermethrin, a 24 month study with **rats** administered cypermethrin in the feed at doses of 0, 0.05, 0.5, 5.0, and 50 mg/Kg bw/d, resulted in 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 females, but no substance related mortality. Some minor fluctuations

were seen in haematological parameters in the interim and 2 year groups, but these were not considered to be of toxicological significance. The 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. In addition, 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. However, increased liver weight was associated with enzyme activity induction and increased kidney weight was 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. 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 revealed no evidence of increased risk of tumour development over the 2 year period (see Section 3.7 for a more comprehensive discussion). The general health and behaviour of control and treated rats were similar throughout the study. Clinical signs were not compound related. The NOAEL in this study was 5 mg/Kg bw/d.

Histopathology: sciatic nerve degeneration: number affected/nurver survivors

Endpoint/dose	0 mg/Kg bw/d		0.05 mg/Kg bw/d		0.5 mg/Kg bw/d		5.0 mg/Kg bw/d		50 mg/Kg bw/d	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
at 12 months	2/12	1/12					0/6	2/6	1/6	2/6
at 18 months	9/24	2/17					4/12	2/10	5/12	0/12
at 24 months	17/31	10/20	8/11	3/8	11/13	4/9	10/17	5/9	12/17	5/12
Total	28/67	13/49					14/35	9/24	18/35	7/30
%	42	26.5					40	37.5	51.4	23

Non-rodent

A waiver argument for the second non-rodent species chronic toxicity was submitted by the applicant and was considered acceptable by the RMS.

From the results of the chronic toxicity study in the rat and comparing this against the 90-day subchronic toxicity study, there is no apparent change in the actual No Observed Adverse Effect Levels (see Doc IIIA6.4.1_01 and Doc IIIA6.5/6.7). This suggests that there is no increase in the overall toxicity of the material following prolonged dosing. It is seen, from the Absorption, Distribution and Excretion study in the rat (Doc IIIA6.2_01) that repeated exposure to the test material over 10 days does cause an increase in blood and tissue concentrations (particularly inguinal and perirenal fat). This increase in concentrations does not alter the overall toxicity, when exposure continues over prolonged periods. Clearance is also fairly rapid following cessation of treatment.

The results of the subchronic repeat dose study in the dog (Doc IIIA6.4.1_02) also shows similar target organ toxicity to that observed in the rat. There was no significant reduction in the No Observed Adverse Effect Level for the dog subchronic study when compared to the equivalent rat study. For these reasons, and to minimise any unnecessary animal testing, it is concluded that sufficient data has been

collected from the existing studies to enable a suitable prediction of the adverse effects of chronic exposure to the test material.

Conclusion on repeated dose toxicity: (from Doc. III-A 6.3, 6.4 and 6.5)

The **short-term dermal toxicity** of cypermethrin was studied in a 21-day dermal toxicity study in rabbits. This resulted in irritation of the skin and was associated to systemic effects such as focal liver necrosis. NOAEL = 20 mg/Kg bw/d.

The **short/medium-term oral toxicity** of cypermethrin was studied in rats and dogs. The central nervous system and the liver were detected as the target tissue/organ. Neurotoxicity was characterised by clinical signs including piloerection, nervousness and uncoordinated movements, ataxia, splayed gait and hyperesthesia. In the dog, clinical signs of neurotoxicity were observed at 37.5 mg/Kg bw/d in a 90-day study (NOAEL = 12.5 mg/Kg bw/d). In the rat, clinical signs of neurotoxicity were observed at 80 mg/Kg bw/d in a 90-day study (NOAEL = 20 mg/Kg bw/d). In rats, neurotoxicity was confirmed by histopathology by peripheral nerve damage. (not in dogs). In addition, body weight was reduced, liver weight increased, and rats presented signs of anemia. In the open literature liver toxicity was characterised by inhibition of the rat liver ATPase activity. The oxidative stress induced by cypermethrin in the cerebral and hepatic tissues was evidenced by enhanced lipid peroxidation. Additionally, a decrease in delayed type hypersensitivity, leucopenia and immunotoxicity were observed when rats were dosed cypermethrin orally for 90 days at doses of 40 mg/Kg bw/d (NOAEL = 10 mg/Kg bw/d.

The **long-term oral toxicity** of cypermethrin was studied in rats. The effects were in line with those observed in the medium-term studies. The central nervous system, liver, and kidneys were detected as the target tissues/organs. Hepatotoxicity was characterised by increased liver weight associated with microsomal enzyme activity induction, but not associated with histological lesions. Increased kidney weight was associated with an increase in blood urea.

Classification/Labelling of the active substance 'cypermethrin' for repeated-dose toxicity according to the criteria in Directive 67/548/EEC and agreed at the 29th ATP: **None**

Classification/Labelling of the active substance 'cypermethrin' for repeated-dose toxicity according to the criteria in CLP-Regulation (EC) No 1272/2008: **STOT RE2; H373. May cause damage to organs through prolonged or repeated exposure.**

This proposal still has to be validated by ECHA.

3.6 GENOTOXICITY

3.6.1 In vitro

Table 3.6.1.: In-vitro genotoxicity of cypermethrin

Test system Method Guideline	Organism/ strain(s)	TS	Test Concentrations	Result		Remark	Reference
				+ S9	- S9		
Gene mutation in bacteria Bactierial reverse mutation test OECD 471	Salmonella typhimurium : TA 98, TA 100, TA 1535, TA 1537. E. coli: WP2 uvr A	Cypermethrin *cis:trans*/40:60, 94.4%	4.88, 9.75, 19.5, 39, 78, 156 µg/plate with and without S9 mix solvent: DMSO positive controls	-	-	Cytotoxic concentration > 1250 µg/plate (preliminary study) Precipitation evident ≥ 156 µg/plate No mutagenic activity ≤ 156µg cypermethrin/plate The positive controls performed appropriately	Oláh (1999a)
Cytogenetic study in mammalian cells In vitro mammalian chromosome aberration test OECD 473	CHOcells, subline KI	Cypermethrin *cis:trans*/40.2:59.8, 93.6%	10, 50, 100 µg/ml with and without S9 mix solvent: DMSO positive controls	-	-	No significant increase in the number of aberrations without gaps with and without metabolic activiation. Both positive controls showed a clear clastogenic effect. In the untreated control, the number of aberrations without gaps was <5%. Non- clastogenic	Oláh (2002)
Gene mutation in mammalian cells Mouse lymphoma assay OECD 476	L5178Y TK+/- mouse lymphoma cells	Cypermethrin *cis:trans*/40:60, 93.6%	Experiment 1: 65 to 2080 µg/ml with and without S9 mix Experiment 2: 2.03 to 260 µg/ml without S9 16.25 to 2080 µg/ml with S9 solvent: DMSO positive controls	-	-	No significant increase in mutant frequency at the TK+/- locus with and without metabolic activation. Neither of the vehicle control mutant frequency values were outside the acceptable range of 50 to 200 x 10-6 viable cells. Both of the positive controls produced marked increases in the mutant frequency per viable cell. Non-mutagenic	Flanders (2011)
Gene mutation in bacteria Plate incorporation assay, Fluctuation test	Salmonella typhimurium : TA 98, TA 100	Cypermethrin, 97%	Plate incorporation test: 0, 30, 100, 300, 1000 µg/plate. Fluctuation test: 0, 1, 3, 10 µg/ml Solvent: DMSO Positive controls	- -	- -	No mutagenic activity in the plate incorporation assay or in the fluctuation test. Precipitation evident ≥ 1000 µg/plate Weakness: only 2 strains tested;	Pluijmen et al. (1984)
Gene mutation in mammalian cells Induction of OUA^r and TG^r	V79 chinese hamster cells	Cypermethrin, 97%	0, 2, 10, 20 µg/ml With and without rat hepatocytes Positive controls	-	-	Not mutagenic for either genetic locus in V97 cells	Pluijmen et al. (1984)

Cytogenetic study in mammalian cells Chromosomal aberration test, Sister Chromatid Exchange	Cultured primary mouse spleen cells	Cypermethrin 1:1, 93%	0, 0.25, 0.5, 1.0, 4.0 µg/ml vehicle: ethanol positive control			Potent inducer of metaphases with chromosome aberrations at all doses after 4h., dose-dependent increase. Tetraploid metaphases observed. Dose-dependent increase in frequency of SCEs. Cell viability: 87.4-99.9% compared with control	Amer et al. (1993)
Cytogenetic study in mammalian cells *In vitro* micronucleus test: cytokinesis-block method	Whole blood (3 donors) Human lymphocytes (2 donors)	Cypermethrin 90%	0, 10, 25, 50, 100, 200 µg/ml vehicle: DMSO positive control: MMC 0.4µM. Cytochalasin B: 6 µg/ml		-	No dose-dependent increase in MN frequencies. Dose-dependent cytotoxicity. Whole blood: The positive results reported at 200 µg/ml are associated with a reduced proliferation index of 36% and 44% respectively.	Surrallés et al. (1995)
Cytogenetic study in mammalian cells Chromosomal aberration test, Sister Chromatid Exchange	Human peripheral blood lymphocytes	Cypermethrin 95%	0, 2, 4, 10, 20, 40, 50 µg/ml^ Solvent: DMSO No positive control		-	Negative: no increase in frequencies of chromosome-type aberrations and sister chromatid exchanges. At 50 µg/ml: mitotic index reduced of 80%.	Puig et al. (1989)
Cytogenetic study in mammalian cells Alkaline comet assay	Human peripheral blood lymphocytes (1 donor)	Cypermethrin 97.1%	0, 10, 50, 100, 200 µg/ml for 0.5h at 37°C solvent: DMSO positive control: hydrogen peroxide 100 µM triplicate experiments			200 µg/ml: increased DNA damage: Tail length: ↑ at 50 and 200 µg/ml Tail moment: ↑ 200 µg/ml Tail intensity: ↑ 10 and 200 µg/ml No cytotoxicity observed.	Ündeğer and Başaran (2005)
Cytogenetic study in mammalian cells 1) Cytotoxicity /vialibility: MTT assay 2) Chromosomal analysis: Chromosomal Aberration test 3) DNA damage: Alkaline comet assay	Human peripheral blood lymphocytes	Cypermethrin	1) 21.6 to 45.6 µM (steps of 2.4µM) 2) and 3) 0, 3.36, 3.60, 3.84 µM Solvent: DMSO			1) Dose-dependent cytotoxic effect. Calculated LC50: 33.6 µM. 2) Frequency of chromatid breaks: 3-4%; Satellite associations: 4-5%; Aneuploidy: 2% 3) dose-dependent increase in DNA single strand breaks. tail length of control: 3.299 µm; 3.36 µM: 12.192 µm; 3.60 µM: 21.242 µm; 3.84 µM: 26.678 µm.	Suman et al. (2006)

Cytogenetic study in mammalian cells Determination of DNA-protein crosslinks, non-DPC DNA adducts, DNA interstrand crosslinks	Mouse hepatocytes Calf thymus DNA	Cypermethrin 94%	0, 0.78, 3.13, 12.5, 50, 200 µg/ml with/without SKF-525A Solvent: DMSO Positive control: formaldehyde Three replicates per treatment			↑ non-DNA-protein croslinks DNA adducts, DNA interstrand crosslinks: ↑ in ratio of DNA of mouse hepatocytes without the cytochromeP450 inhibitor.	Cui et al. (2006)
Clastogenicity: modified Allium test	Allium cepa root tip cells	Cypermethrin, no more data	0, 0.1, 1, 10 µg/ml solvent: ethanol			Toxic effects: stickiness of chromosomes and weak C-mitosis effect Mitotic activity affected at 10 µg/ml	Kumar et al. (2004)

A **gene mutation** assay in **bacterial cells** was submitted (Oláh, 1999a). Cypermethrin cis:trans/40:60 dissolved in DMSO was tested for mutagenic activity with the reverse mutation assay. The experiments were carried out twice with *Salmonella typhimurium* strains TA98, TA100, TA1537, TA1535 and the *Escherichia coli* strain WP2 uvrA with and without metalolic activation. There was precipitation in the solution with ≥ 156 µg cypermethrin/2 ml top agarconcentration and no precipitation in the solution with ≤ 78 µg cypermetrhin/2 ml top agar concentration. Cypemethrin was tested at concentrations of 156, 78, 39, 19.50, 9.75, and 4.88 µg/plate. In both the preliminary study and the main studies the colony numbers in control (solvent, untreated) plates and test plates was practically the same. Cypermethrin showed no mutagenic activity (≤ 156 µg/plate). Positive controls performed appropriately.

Recently a **mammalian gene-mutation** assay was submitted (Flanders, 2011). The study was conducted according to the method that was designed to assess the potential mutagenicity of the test item on the thymidine kinase, TK +/-, locus of the L5178Y mouse lymphoma cell line. Two independent experiments were performed. In Experiment 1, L5178Y TK +/- 3.7.2c mouse lymphoma cells were treated with cypermethrin at six dose levels, in duplicate, together with vehicle (solvent: DMSO) and positive controls using 4-hour exposure groups both in the absence and presence of metabolic activation (2% S9). In Experiment 2, the cells were treated with cypermetrhin at eight dose levels using a 4-hour exposure group in the presence of metabolic activation (1% S9) and a 24-hour exposure group in the absence of metabolic activation. The dose range was selected following the results of a preliminary toxicity test and for the first experiment was 65 to 2080 µg/ml in both the absence and presence of metabolic activation. For the second experiment the dose range was 2.03 to 260 µg/ml in the absence of metabolic activation, and 16.25 to 2080 µg/ml in the presence of metabolic activation. The maximum dose level used for the 4-hour exposure groups in the mutagenicity test was limited by a combination of toxicity and the presence of precipitate effectively reducing exposure of cypermethrin to the cells, the maximum dose level in the 24-hour exposure group was limited by toxicity. The vehicle (solvent) controls had acceptable mutant frequency values that were within the normal range for the L5178Y cell line at the TK +/- locus. The positive controls induced marked increases in the mutant frequency indicating the satisfactory performance of the test and of the activity of the metabolising system. Cypermethrin did not induce any toxicologically significant dose-related increases in the mutant frequency at any dose level, either with or without metabolic activation, in either the first or the second experiment. Cypermethrin cis:trans/40:60 was non-mutagenic to L5178Y mouse lymphoma cells.

Summary of results – experiment 1:

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

Summary of results – experiment 2:

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

%RSG = Percentage Relative Suspension Growth; RTG = Relative Total Growth; MF = Mutation Frequency

In addition an *in vitro* **mammalian chromosomal aberration** study was submitted (Oláh, 2002). Cypermethrin cis:trans/40:60 at concentrations of 10, 50, and 100 µg/ml, dissolved in DMSO, was studied for clastogenic activity in CHO cells both in the presence and absence of metabolic activation. Two separate experiments were performed with an exposure period of 4 hours and 1.5 normal cell cycle length respectively (18-20 hours). There was no increase in the number of aberrations without gaps with and without metabolic activation in either experiment. In the untreated control the number of aberrations without gap was less than 5% proving the suitability of the usd cell line. The positive controls, ethyl methanesulphonate and N-Nitrosodimethylamine, showed a clear clastogenic effect thereby validating

the test. Cypermethrin cis:trans/40:60 did not induce chromosomal aberrations and proved to be non-clastogenic in Chinese Hamster ovary cells.

Conclusion on submitted regulatory studies:

A well-performed **bacterial reverse gene mutation** assay, both with and without S9, was found negative. Cypermethrin was found negative for mutagenicity in the ***in vitro*** **gene mutation assay in mammalian cells** (L5178Y mouse lymphoma cells)In addition, a well-performed ***in vitro*** **mammalian chromosomal aberration** study on CHO-cells was provided. Under these conditions, cypermethrin did not induce chromosomal aberrations in cultured CHO-cells. In conclusion, cypermethrin was found negative for genotoxic effects in *in vitro* bacterial and mammalian cells test systems.

The open literature

Additional information concerning the *in vitro* genotoxicity potential of cypermethrin was found in the open literature. The data reported on the genotoxicity of cypermethrin are rather inconsistent, depending on the genetic system or the assay used.

The negative outcome in the gene-mutation tests was confirmed in bacterial cells (± S9) and V79 cells in the presence or absence of rat hepatocytes by Pluijmen et al. (1984).

The potential to induce chromosomal aberrations was tested in mouse spleen cells, mouse hepatocytes and in human peripheral blood lymphocytes. In cultured mouse spleen cells, cypermethrin was found to be a potent inducer of metaphases with chromosomal aberrations at all doses at 4 h (dose-dependent) after excluding the metaphases with chromosome and chromatid gaps. Tetraploid metaphases were observed in the treated cell cultures (Amer et al., 1993). Of note, ethanol (vehicle) also induced a significant increase in chromosome aberrations. In human peripheral blood lymphocytes, the chromosomal aberration test was found negative by Puig et al. (1989). Nevertheless, aneuploidy was shown by Suman et al. (2006), and karyotype analysis revealed more satellite associations and chromosomal breaks in cypermethrin treated samples. Cui et al. (2006) demonstrated the potential of cypermethrin to form non DNA protein crosslinks and DNA interstrand crosslinks in mouse hepatocytes without cytochrome P450 inhibitor whereas with the inhibitor or in calf thymus DNA cypermethrin could not form DNA monoadducts and DNA interstrand crosslinks. It appears that cytochrome P450 may be involved in activation of cypermethrin, and as such, the active metabolites of cypermethrin, instead of cypermethrin itself, cause DNA interstrand crosslinks (Cui et al., 2006).

Cypermethrin was also tested in the SCE test system. Contradictory results were obtained. In cultured mouse spleen cells, a dose-dependent increase in frequency of SCEs was observed (Amer et al., 1993) whereas in human peripheral blood lymphocytes no increases were found (Puig et al., 1989).

In an *in vitro* micronucleus test conducted on human whole blood and cultured human lymphocytes no dose-dependent induction of micronuclei was found. The positive results reported at 200 µg/ml were associated with a reduced proliferation index. Therefore, it was concluded that the *in vitro* micronucleus test produced negative results (Surrallés et al., 1995).

The alkaline comet assay performed on human peripheral blood lymphocytes was found positive in 2 studies. Increased DNA damage was observed at 200 µg/ml (increase in tail moment) by Ündeğer and Başaran (2005). A dose-dependent increase in DNA single-strand breaks, assessed by measuring tail length of comets, was also found by Suman et al. (2006).

3.6.2 In vivo

Table 3.6.2.: In vivo genotoxicity of cypermethrin

<table>
<tr><th>Type of test
Method/
Guideline</th><th>TS</th><th>Species
Strain
Sex
no/group</th><th>Frequency of application</th><th>Sampling times</th><th>Dose levels</th><th>Results</th><th>Remarks</th><th>Reference</th></tr>
<tr><td>Mouse bone marrow micronucleus test
OECD 474</td><td>Cypermethrin cis:trans/40:60, 94.4%</td><td>NMRI mice
m/f
5/sex/ group</td><td>Single oral
(Positive control: ip)</td><td>24, 48 hrs after oral administra-tion
(pos.control: 48h; untreated: 24h)</td><td>50, 75, 100 mg/Kg bw
Vehicle: sunflower oil</td><td>Single doses of 50, 75, 100 mg/Kg did not induce an increase in the frequency of MVPEs in ♀&♂ at 24h , compared with the vehicle control. 48h after treatment: MCPEs number ↑ in ♂ at 50 and 100 mg/Kg, and in ♀ at 75 mg/Kg. Biological relevance is questionable.
PCE/NCE ratios males:
<table>
<tr><th></th><th colspan="2">PCE/NCE ratio</th></tr>
<tr><td></td><td>24h</td><td>48h</td></tr>
<tr><td>Un-treated control</td><td>1.27</td><td>-</td></tr>
<tr><td>Pos control</td><td></td><td>0.31</td></tr>
<tr><td>Veh control</td><td>1.18</td><td>1.18</td></tr>
<tr><td>50 mg/Kg</td><td>1.12</td><td>1.08</td></tr>
<tr><td>75 mg/Kg</td><td>1.14</td><td>1.11</td></tr>
<tr><td>100 mg/Kg</td><td>1.08</td><td>1.07</td></tr>
</table>
PCE/NCE ratios females:
<table>
<tr><th></th><th colspan="2">PCE/NCE ratio</th></tr>
<tr><td></td><td>24h</td><td>48h</td></tr>
<tr><td>Un-treated control</td><td>1.24</td><td>-</td></tr>
<tr><td>Pos control</td><td></td><td>0.34</td></tr>
<tr><td>Veh control</td><td>1.21</td><td>1.25</td></tr>
<tr><td>50 mg/Kg</td><td>1.13</td><td>1.19</td></tr>
<tr><td>75 mg/Kg</td><td>1.18</td><td>1.11</td></tr>
<tr><td>100 mg/Kg</td><td>1.11</td><td>1.07</td></tr>
</table>
Significant depression of PCE/NCE ratio was not observed
Conclusion: negative for mutagenicity in NMRI mice</td><td>MTD = 100 mg/Kg bw determined in range finding study.</td><td>[redacted]</td></tr>
</table>

Mouse bone marrow micronucleus test OECD 474	Cypermethrin *cis:trans*/40:60, 93.5%	Swiss mice m/f 5/sex/ group	Single oral (1 high dose groupand the corresponding negative control received 2 doses on consecutive day swith 24h interval)	24, 48 hrs after oral administra-tion (pos.control: 48h; untreated: 24h and 48h, and 24h after second treatment of 1 group; 1 high dose group 24h after second administration)	12.5, 25, 50 mg/Kg bw Vehicle: corn oil	Cypermethrin did not induce a significant increase in the number of micronucleated PCEs in males and females at any dose level 24 or 48 h after single treatment, or 24 h after 2 administrations. The mean percentage of micronucleated PCEs in the treated groups was consistently lower than the vehicle mean for males and females at the 24 h and 48 h t points in the low and intermediate groups and the high dose groups were similar to the controls. PCE:NCE ratios negative control: 24 h males and females: 1.35 and 1.42 48 h males and females: 1.37 and 1.43 24 h after two treatments: 1.47 and 1.45 PCE:NCE ratios positive controls: 24 h males and females: 1.75 and 1.83 PCE:NCE ratios low and intermediate dose treated group: 24 or 48 h males and females: in range of 0.86 to 1.28 (indicating a slight reduction in the ration in comparison with controls) PCE:NCE ratios high dose treated group: 24 or 48 h males and females: in range of 0.69 to 0.85 after one or two administrations (indicating a significant reduction in the PCE:NCE ratio). There were no marked differences in the frequencies of micronucleated polychromatic erythrocytes for either sex in any of the treatment groups. Positive control:, sign. increase in the number of micronucleated PCEs 24 h after application.	MTD = 50 mg/Kg bw determined in range finding study.	[redacted]

Mouse bone marrow micronucleus assay / no guideline specified	Cypermethrin 93%	Swiss mice m/f 2-3 mice/dose	1) intraperitoneal single: 180 mg/Kg bw; repeated: 60 mg/Kg bw, twice weekly, 3 injections in total; solvent: DMSO 2) oral: diet 300, 900 ppm for 1, 7, 14 consecutive days; solvent: acetone 3) dermal 120, 360 mg/Kg bw , twice weekly for 2 weeks; solvent: DMSO No positive control	1) 1, 2, 7 days after single injection; weekly after repeated injection 2) 24 h, 14 d recovery 3) 24h, 14 d recovery		1) Onset of toxic signs was rapid and disappeared in survivors after a few days. Increased ratio of PCEs to NCEs for 3 x i.p. 60 mg/Kg bw. No increase in the number of polychromatic erythrocytes (PCEs) containing micronuclei (MN) 2) Normal growth, no mortality, no clinical signs mentioned. 900 ppm, 7 and 14 d treatment, sampling 24h: increased PCEs containing MN. 3) Acute toxicity: at 360 mg/Kg bw, mortality rapid, 20%. Toxic to the bone marrow. Sampling at 24h: 2 and 4 treatments induced an increase in PCEs containing MN.	Oral: not by gavage, but via the diet; no positive control used; only 1 sampling time.	[redacted]
Bone marrow chromosome aberration test	Cypermethrin, technical grade	Swiss mice 3/group	Acute: ip, po, sc	6, 24, 48 h after dosing	30, 40, 50 mg/Kg bw solvent: DMSO	Both chromatid and chromosome type aberrations. Chromatid type aberrations included gaps and deletions such as breaks, fragments, rings; chromosome type aberrations included deletions such as minutes, exchanges leading to metacentric chromosomes. Gaps more frequent than deletions.	i.p., acute, all doses, 24h, 48h: dose-dependent increase in aberration frequence; repeated: less but still increased p.o., 50 mg/Kg bw, 24h: increased aberration frequence s.c.: not sign.	[redacted]
			Repeated ip administration for 5 days	120h after first dosing	5 x 10 mg/Kg bw			
Mouse bone marrow micronucleus assay / no guideline specified	Cypermethrin, technical grade	Swiss mice 3/group	2 i.p. injections within 24 hrs	6h after last treatment	30, 40, 50 mg/Kg bw solvent: DMSO	Dose-dependent significant ($p < 0.05$) increase in micronuclei frequency in the bone marrow cells. Micronucleated PCEs > micronucleated NCEs.	No positive control, 6h: short sampling time	[redacted]

Chromosome aberration in mouse spleen and bone marrow	Cypermethrin 1:1, 93%	Swiss male mice 5/group	i.p., single dose	6h, 24 h, 48h after treatment	180 mg/Kg bw solvent: DMSO	Increase in chromosomal aberrations both in spleen and bone marrow 6h after treatment, decreasing with time (after exclusion of metaphases with gaps). Induction of structural and numerical (tetraploid metaphases) chromosomal aberrations.	No positive control	[redacted]
Chromosome aberration in mouse bone marrow	Cypermethrin (Cyperkill 25 EC)	Male mice 5/group	Single oral gavage	24h after trreatment	0.66, 1.32, 2 mg/Kg bw vehicle: peanut oil pos control: cyclophosphamide i.p.	Mitotic Index inhibited at 2 mg/Kg bw, but no induction of chromosome aberrations. Frequent aberrations: chromatid breaks and fragments.		[redacted]
Chromosomal aberration in rat bone marrow	Cypermethrin *cis:trans*/40.1:57, 97.1%	Male Wistar rats	Gavage, 5 times per week, for 4 weeks	24h after last administration	5.54, 11.08, 22.16 mg/Kg bw vehicle: sunflower oil	Cypermethrin did not induce chromosomal aberrations in mouse bone marrow cells after subchronic oral treatment with doses corresponding with 1/100, 1/50, 1/10 LD50	No clinical signs of toxicity reported. Weight gain: not affected Rel spleen weight: low dose ↑ or ↓?	[redacted]
Mouse bone marrow micronucleus assay	Cypermethrin (Cyperkill 25 EC)	Male mice 5/group	Single oral gavage	48h after treatment	0.66, 1.32, 2 mg/Kg bw vehicle: peanut oil pos control: cyclophosphamide i.p.	No increase in micronucleated PCEs		[redacted]
Sister Chromatid Exchange in mouse bone marrow	Cypermethrin 1:1, 93%	Swiss male mice 5 or 6/group	i.p., single dose	24 h after treatment	180, 200, 300 mg/Kg bw solvent: DMSO	Dose-dependent increase in SCE frequency		[redacted]

Sister Chromatid Exchange in mouse bone marrow	Cypermethrin (Cyperkill 25 EC)	Male albino mice 4/group	Single gavage	24 h after administra-tion	10.6, 21.2, 32 mg/Kg bw vehicle: peanut oil pos control: cyclophosphamide	Modest induction in SCE (less than doubling over control rate) at highest dose (clinical signs: transient muscle incoordination, diarrhoea)	No linear relationship between SCE-induction and test doses: possible influence of cell cycle delay/mitotic inhibition by cypermethrin?	[redacted]
Sister Chromatid Exchange in mouse bone marrow	Cypermethrin, 99%	Swiss mice m/f 3/group	i.p., single dose	24h after administration	5, 10, 20 mg/Kg bw control: saline	Increase in frequency of SCEs at all doses, no dose-response correlation, no induction of cell cycle delay	No positive control used	[redacted]
DNA damage in organs and tissues Comet assay	Cypermethrin technical grade, 98.5%	Male Swiss mice 4/group	i.p., 5 consecutive days	6h after last administration	0, 12.5, 25, 50, 100, 200 mg/Kg bw vehicle: corn oil	Dose-dependent increase in DNA damage in all organs and tissues: ↑ in Olive tail moment (not sign. at 12.5 mg/Kg bw). Level of DNA damage: brain > spleen > kidney > bone marrow > liver > lymphocytes Systemic genotoxicity	Pos. control: ethylmethane sulfonate (100 mg/Kg bw, 24h before sacrifice) Organs/tissues tested: bone marrow, brain, kidney, liver, spleen, lymphocytes. Cell viability: > 90% in all experiments	[redacted]
DNA damage in lymphocytes Comet assay	Cypermethrin *cis:trans*/37.2:62.8,	Male Wistar rats 15/group	Gavage for 60 days		0, 2.5, 25 mg/Kg bw /d vehicle: corn oil	No clinical signs of poisoning found. No rat lymphocyte DNA damage up to a 1/10 LD50 dose: tail length, tail intensity, and tail moment not changed.	No positive control	[redacted]

DNA damage in Drosophila melanogaster Comet assay	Cypermethrin98.5%	Drosophila melanogaster, larvae	Feeding	96 h after start feeding	0.0004, 0.0008, 0.002, 0.2, 0.5 ppm vehicle: DMSO	Larvae: increase in DNA damage in the cells of brain ganglia and anterior mid gut Brain ganglia and anterior midgut: sign ↑ tail moment, tail length, tail DNA(%) at 0.002, 0.2, 0.5 ppm, dose-dependent.	Positive control: EMS Cell viability > 95%	Mukhopadhyay et al. (2004)
Mammalian germ cells: Mouse Dominant lethal Assay	Cypermethrin 10% EC	Male Swiss mice 10/group	Single gavage		20, 40, 80 mg/Kg bw vehicle: corn oil	Total implants dose-dependent decreased in first weeks: oligospermic effect? Living implants decreased and status dead implants/female increased in the first (40, 80 mg/Kg bw) and 2nd week (80 mg/Kg bw). Dead implants: late deaths probably resulting from hemopoietic failure. Ratio mean number corpora lutea/female and total number implants/female not elevated. No pre-implantation losses. High rate of post-implantation loss Average Mutagenic Index of 6 weeks: ↑ at 80 mg/Kg bw. Dominant lethal mutation increased the first week (40 mg/Kg bw) and first 3 weeks (80 mg/Kg bw).	Positive control: benzo(a)pyrene (100 mg/Kg bw) Post-implantation losses at 40 and 80 mg/Kg bw during the initial weeks: meiotic clastogenic events in the spermatozoa and the spermatid stages of the spermatogenetic cycle.	[redacted]

Drosophila 1) Sex-linked recessive lethals (SLRL) 2) Sex-chromosome loss (SCL) 3) Non-disjunction (ND)	Cypermethrin, 95%	Drosophila melanogaster	Adult: feeding and injection Larval: feeding		Adult feeding: 0, 5, 10, 20 ppm; Adult injection: 0, 10 ppm; larval feeding: 0, 25 ppm. solvent feeding: 5% sucrose and ethanol, solvent injection: cotton oil	SLRL: Adult injection at 10 ppm and larval feeding at 25 ppm: increase in frequency of recessive lethals in first brood SCL: Ineffective in producing total or partial chromosome losses (feeding and injection) ND: Ineffective	Cypermethrin was found toxic (25ppm adult feeding = 100 % mortality). As it was not possible to give higher doses than 10 ppm by feeding, cypermethrin did not reach the gonads in sufficient quantities to produce a mutagenic effect clearly detectable in the SLRL assay.	Batiste-Alentorn et al. (1986)
Sperm abnormality assay	Cypermethrin, technical grade	Swiss mice	i.p. on 5 consecutive days	35 d after first injection	30, 40, 50 mg/Kg bw solvent: DMSO	Abnormal head shapes and sizes, including giant sperm and branched sperm. Number of aberrant sperm/100 sperm was sign. increased (marginal difference) for all treated groups compared with control, but no dose-dependency.	No positive control	[redacted]
Sperm abnormality assay	Cypermethrin, no more data	Swiss male mice 5/group	i.p., daily for 5 days		0, 30, 60, 90 mg/Kg bw solvent: DMSO pos Control: cyclophosphamide	Body and testes weight: ↓ bw gain at 60 and 90 mg/Kg bw/d Sperm head morphology: ↑ No of abnormal shape of sperm head at 60 and 90 mg/Kg bw/d, dose-dependent		[redacted]

Two *in vivo* bone marrow micronucleus studies in mice were submitted by the applicant.

The potential mutagenicity of Cypermethrin cis:trans/40:60 was examined in bone marrow of NMRI mice according to OECD test guideline 474 by Oláh (1999b). Cypermethrin was administered orally at 50, 75, or 100 mg/Kg bw. Following a range-finding study, the MTD was found to be 100 mg/Kg bw. In the main study, animals were treated once orally and samples were taken 24 and 48 hours after treatment. During the microscopic evaluation, 2000 PCEs were scored per animal to assess the

micronucleated cells. Cypermethrin did not induce significant increase in the number of micronucleated PCEs in either males or females at any dose level 24 hours after treatment. After 48 hours, significant increases in the number of MPCEs were seen in male mice at the 50 and 100 mg/Kg dose and in females at the 75 mg/Kg dose level. However, the significance of the increases is inconclusive due to the absence of a dose-response relationship. The positive control, cyclophosphamide, caused a significant increase in the number of MPCEs 48 hours after application, thus validating the test. No differences in the ratio of polychromatic and normochromatic erythrocytes were found after treatment and significant depression of the PCE:NCE ratio was not observed. Nevertheless, it is assumed that cypermethrin reached the bone marrow because cypermethrin is well distributed according to the ADME study.

In addition, the potential mutagenicity of Cypermethrin cis:trans/40:60 was examined in bone marrow of Swiss albino mice according to OECD test guideline 474 by [redacted]. The *in vivo* micronucleus assay assessed the clastogenic and aneugenic potential of the test substance, which was administered orally at 12.5, 25, 50 mg/Kg bw. Following a range-finding study, the MTD was found to be 50 mg/Kg. In the main study, animals were treated once via the oral route (corn oil used as the vehicle) and samples were taken 24 and 48 hours after treatment. One group of vehicle controls and a high dose group were treated on two occasions and samples collected 24 hours after the second dosing occasion.

Cyclophosphamide was included as a standard positive control for the assay. During the microscopic evaluation, 2000 PCEs were scored per animal to assess the micronucleated cells. The PCE:NCE ratio was evaluated from assessment of 200 erythrocytes per group. Single oral doses of 12.5, 25, or 50 mg/Kg bw did not induce an increase in the frequency of micronucleated polychromatic erythrocytes (MCPEs) in male and female mice at 24 and 48 hours after treatment when compared to the vehicle control. The positive control induced a statistically significant increase in MPCE thereby validating the techniques used in the assay. The ratio of polychromatic to normochromatic erythrocytes was similar to the vehicle controls for the low and intermediate treatment regimen, although a slight reduction was evident for the treated groups at both sampling time points. The high dose group showed a reduction in the ratio also with a significantly lower ratio at 50 mg/Kg compared to the vehicle control. Cypermethrin technical induced no apparent chromosomal or other change leading to formation of micronuclei in polychromatic erythrocytes at dose levels up to the MTD of 50 mg/Kg. Cypermethrin technical was not considered to be genotoxic and proved to be negative for mutagenicity in the mouse in-vivo bone marrow micronucleus test.

Males

	PCE/NCE ratio		**% MN**	
	24h	48h	24h	48h
Vehicle	1.35	1.37	0.54	0.45
Pos. control	1.01	Na	1.75	na
12.5 mg/Kg	1.11	1.12	0.40	0.42
25 mg/Kg	0.92	1.02	0.42	0.40
50 mg/Kg	0.72	0.76	0.58	0.43

Females

	PCE/NCE ratio		**% MN**	
	24h	48h	24h	48h
Vehicle	1.42	1.43	0.43	0.33
Pos. control	1.10	na	1.83	na
12.5 mg/Kg	1.06	1.28	0.49	0.39
25 mg/Kg	0.98	0.86	0.42	0.34
50 mg/Kg	0.69	0.85	0.55	0.42

Two day treatment –sampled 24 hrs after dosing completed

	PCE/NCE ratio		% MN	
	m	F	m	f
Vehicle	1.47	1.45	0.32	0.35
50 mg/Kg	0.77	0.72	0.40	0.46

Conclusion on submitted regulatory studies:

In vivo, cypermethrin did not produce micronuclei in the immature erythrocytes of 2 well-performed **mouse bone marrow micronucleus assays** (single oral dose), and was, therefore, considered negative for mutagenicity.

The open literature

Additional information concerning the *in vivo* genotoxicity potential of cypermethrin was found in the open literature.

In mouse bone marrow, cypermethrin may induce gross structural chromosomal aberrations (micronuclei) and more discrete lesions (chromosome aberrations) ([redacted]). Structural as well as numerical (tetraploid metaphases) chromosome aberrations were induced in the mouse spleen ([redacted]). Contradictory results were obtained in other studies showing only chromatid breaks and fragments in mouse bone marrow or in rat bone marrow ([redacted]). Induction of SCEs was observed without induction of cell cycle delay in mouse bone marrow, although not always in a dose-dependent way depending on the test protocols used ([redacted]; [redacted]; [redacted]). The comet assay demonstrated DNA damage induced by cypermethrin in all tissues in the mouse with the highest level of DNA damage in the brain and the lowest level in the lymphocytes, suggesting systemic genotoxicity ([redacted]). In Drosophila melanogaster, DNA damage was confirmed in the cells of the brain ganglia and anterior mid gut (Mukhopadhyay et al., 2004). However, in rat lymphocytes, the comet assay was found negative ([redacted]).

A mouse dominant lethal assay performed with cypermethrin resulted in an increase in dominant lethal mutations in the 3 first weeks. The increased post-implantation losses in the initial weeks were suggestive for meiotic clastogenic events in the spermatozoa and the spermatid stages of the spermatogenetic cycle ([redacted]). Two sperm abnormality assays ([redacted]; [redacted]) demonstrated an increase in abnormal shape of the sperm head. The induction of sperm abnormalities indicates that cypermethrin may have potential genotoxic properties in germline cells.

The sex-linked recessive lethals test indicated that cypermethrin acts as a weak mutagen in Drosophila, increasing significantly the frequency of gene mutation (recessive lethals in the first brood), but was ineffective in the tests assaying for chromosomal damage (sex chromosome loss, non-disjunction) (Batiste-Alentorn et al., 1986).

Although the relevance of these open literature studies can be challenged (route of administration deviating from guideline protocols, deviating time of sampling, and other procedural flaws), these studies reveal indications of some non-specific damage (DNA adducts, alkaline DNA fragmentation). Also higher-tier genotoxicity studies (sperm abnormality assay, SLRL in Drosophila) revealed positive results, indicating that the genotoxic effects of cypermethrin in somatic cells was also reproduced in germinal cells.

In conclusion, a wide spectrum of genotoxic effects of cypermethrin is demonstrated. Nevertheless, the molecular mechanisms of the genotoxicity of cypermethrin remain to be elucidated.

Summary of genotoxicity: (from Doc. III-A 6.6)

Cypermethrin was found negative for genotoxic effects in *in vitro* bacterial and mammalian cell test systems (bacterial reverse gene mutation assay, mammalian gene mutation assay in L5178Y mouse lymphoma cells, mammalian chromosomal aberration study on CHO-cells). *In vivo*, cypermethrin did not produce micronuclei in the immature erythrocytes of the mouse bone marrow micronucleus assay (single oral dose), and was, therefore considered negative for mutagenicity.

Overall, the open literature provides inconsistent evidence of genotoxicity *in vitro* as well as *in vivo*. The data reported on the genotoxicity of cypermethrin are rather inconsistent, depending on the genetic system or the assay used. Most of these studies were not performed according to accepted guidelines. Additionally, they lack reliability because of procedural flaws such as deviating route of administration, single versus repeated exposure, other sampling times, no use of positive controls, no 2^{nd} or 3^{rd} confirming experiments, no data about reaching the target organ. Nevertheless, the modest or marginal increases in DNA damage reported in some studies in peripheral lymphocytes or other cells indicate, at least to a limited extent, potential genetic hazards posed by cypermethrin, and emphasize the need and the importance of protective measures and safety regulations to minimize exposure to cypermethrin.

Although the genotoxicity studies on cypermethrin did not exclude a potential for DNA damage, the global weight-of-evidence suggests that cypermethrin should not be considered a genotoxicant, and thus no DPD classification as a Category 3 mutagen is warranted, nor a CLP classification is foreseen.

In addition, there was no evidence of carcinogenicity in the rat. Also in other repeated-toxicity studies, there was no evidence of proliferative lesions, which would possibly occur if cypermethrin would display aneuploidogenic or polyploidogenic behaviour *in vivo*.

Classification/Labelling of the active substance 'cypermethrin' for genotoxicity according to the criteria in Directive 67/548/EEC: **None**

Classification/Labelling of the active substance 'cypermethrin' for genotoxicity according to the criteria in CLP-Regulation (EC) No 1272/2008: **None**

3.7 CARCINOGENICITY

Table 3.7.: Carcinogenicity of cypermethrin

Route	Method Guideline	TS	Species Strain Sex no/group	Dose levels frequency of application	Tumors	Reference
Oral feed	Deviating OECD 453 Deviations: low number of rats; blood albumin, glucose, glucose, GGT, ornithine decarboxylase not measured; urinalysis not performed.	Cypermethrin *cis*:*trans*/1:1, 97% (WL 43467)	Wistar rats m/f 24/sex/group	0, 1, 10, 100, 1000 ppm (0, 0.05, 0.5, 5, 50 mg/Kg bw/d) Daily over 24 months (combined chronic toxicity / carcinogenicity study)	The NOAEL in this study was 100 ppm, equivalent to 5 mg/Kg bw/d in male and female rats. **Overview of tumours found**: Pituitary: Anterior lobe adenoma, anterior lobe carcinoma, intermediate lobe adenoma, posterior lobe-asrrocytoma; Mammary glands: Fibroadenoma, adenocarcinoma; Uterus: Adenocarcinoma, endometrial sarcoma. The most common tumour was **pituitary adenoma**, which occurred in 39 of 48 controls in the 2 year group and was the cause of early death or removal from the trial in 19 of these. Similar incidences were found at other dose levels in females. These were present, but much less common, in males and were believed not to be treatment-related. A cluster of 4 uterine tumours in the 5 mg/Kg bw group was reported. Since there were no uterine tumours at 50 mg/Kg bw and there was no question of competing toxicity at 50 mg/Kg bw preventing their development, the uterine tumours at 5 mg/Kg bw were not considered to be compound related. Statistical analysis revealed no evidence of increased risk of tumour development following dietary inclusion of cypermethrin for up to 2 years in rats. Cypermethrin was found **not carcinogenic** in this study.	[redacted]

Comparative overview and incidence of tumours found by site, type, %

Tissue/pathological finding % affected animals	**0 mg/Kg bw/d**		**0.05 mg/Kg bw/d**		**0.5 mg/Kg bw/d**		**5 mg/Kg bw/d**		**50 mg/Kg bw/d**	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Pituitary										
anterior lobe adenoma (TA) (B)	33	79	20	91	29	100	20	79	25	75
anterior lobe carcinoma (M)		6.2	4.		4.1		4.1		4.1	
intermediate lobe adenoma (B)					4.1					
posterior lobe-asrrocytoma (M)	2.08								4.1	
Mammary glands										
fibroadenoma (B)		14				33		16		
adenocarcinoma (M)		28		63		33		16		40
Uterus										
adenocarcinoma (M)				4.1				4.1		
endometrial sarcoma (M)								8		

(B) benign; (M) malignant

Route	Method Guideline	TS	Species Strain Sex no/group	Dose levels frequency of application	Tumors	Reference
Oral feed	Medium-term liver bioassay	Cypermethrin *cis:trans*/52:48	F344 rat male	1) single i.p. Diethylnitrosamine (DEN) 200 mg/Kg bw, after 2 weeks recovery: cypermethrin 800 ppm for 6 weeks, after 1 week: 2/3 partial hepatectomy 2) same as 1) but without treatment with cypermethrin 3) same as 1) but single i.p. with 0.9% saline	**General:** Cypermethrin did not affect either body or liver weight gains with the dose of 800 ppm (48 mg/Kg bw/d) for 6 weeks. **Number and area of GST-P positive foci per unit area of liver section**: not increased in cypermethrin treated animals. Also without the DEN initiation, cypermethrin did not induce GST-P positive liver cell foci.	[redacted]
Oral feed	No guideline Induction of altered hepatic foci in vivo	Cypermethrin, 90%	Sprague-Dawley rat male	500 or 1000 ppm for 20 weeks and this after a two weeks recovery following a single i.p. injection of N-nitrosodiethylamine 30 mg/Kg bw and partial hepatectomy	In initiated rats, cypermethrin induced an increase in the number of foci per liver and in the percent tissue occupied by GGT-positive foci. Cypermethrin did not enhance the volume fraction of the liver occupied by GST-P positive foci. Without initiation, no such foci were found. Cytochrome P 450-related O-dealkylase enzyme activities in hepatic S9 fractions (EROD, PROD) showed that cypermethrin only marginally (but significantly) induced EROD and PROD. Elevated liver weight seemed not to be correlated to cytotoxicity and necrosis: no pathological alterations were seen, transaminase activities (ALT, AST) not consistently increased. In conclusion: Cypermethrin can act as a tumour promoter.	[redacted]

Dermal	Mouse skin model for carcinogenesis	Cypermethrin 10% EG (Bilsyp)	Swiss mice m/f 20/sex/group	*Bioassay carcinogenic potential:* 5, 10, 25 mg/Kg bw 3/week for 52 weeks. Untreated control, vehicle control (aceton) and pos control (BaP) included. *Bioassay tumour initiating potential:* 10 mg/Kg bw single or 3/week for 3 weeks followed 1 week later by 5 µg TPA in acetone. Untreated, vehicle (acetone) and pos control (DMBA). *Bioassay tumour promoting potential:* 1 week after initiation with DMBA topical application of 10 mg/Kg bw cypermethrin 3/week for 32 weeks, vehicle: acetone, pos control: TPA	*Bioassay complete carcinogenic potential:* Induction of tumour formation in ♂ and ♀ at all doses tested. Animals developing skin tumours at end of study: 2/15, 3/11, 4/11 in ♂, and 2/16, 4/16, 2/12 in ♀. Average number of tumour/mouse of 1.0, 2.0, 3.3 in ♂, and 1.5, 2.3, 3.8 in ♀ with increasing dose. No tumours found in internal organs. Skin tumours: benign, squamous epithelial cells arranged in an arboreal pattern around the inner vascular fibroitic core, designated as squamous cell papillomas. (high incidence of mortality at the high dose group in both ♂ and ♀) *Bioassay tumour initiating potential:* Single dose, initiated mice at endpoint 32 weeks: 9/12 surviving ♂ and 10/14 surviving ♀ developed benign tumours. Multipe dose, initiated mice at endpoint 32 weeks: 7/9 surviving ♂ and 10/13 surviving ♀ developed tumours at application site. Tumour development after 16 (♂) and 17 (♀) weeks. Histopathology on tumours: benign. *Bioassay tumour promoting potential:* Induction of tumour incidence by cypermethrin after DMBA initiation: 4/10 surviving ♂ and 5/13 surviving ♀. Tumour development after 17 (♂) and 19 (♀) weeks. Histopathology: benigne, including pedunculated squamous cell papillomas, flat papillomas and keratoacanthomas.	[redacted]

Rodent

Considering the chronic toxicity/carcinogenicity of cypermethrin, a 24 month study with **rats** administered cypermethrin in the feed at doses of 0, 0.05, 0.5, 5.0, and 50 mg/Kg bw/d (McAusland et al., 1978), resulted in 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 females, but no substance related mortality. Increased liver weight was associated with enzyme activity induction and increased kidney weight was 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. 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. The general health and behaviour of control and treated rats were similar throughout the study. Clinical signs were not compound related. The NOAEL in this study was 5 mg/Kg bw/d. (see Section 3.5 for a more comprehensive discussion).

An overview and the incidence of the tumours found is given in Table 3.7. The most common tumour was **pituitary adenoma**, which occurred in 39 of 48 controls in the 2 year group and was the cause of

early death or removal from the trial in 19 of these. Similar incidences were found at other dose levels in females. These were present, but much less common, in males and were believed not to be treatment-related. A cluster of 4 **uterine tumours** in the 5 mg/Kg bw group was reported. Since there were no uterine tumours at 50 mg/Kg bw and there was no question of competing toxicity at 50 mg/Kg bw preventing their development, the uterine tumours at 5 mg/Kg bw were not considered to be compound related. Statistical analysis revealed no evidence of increased risk of tumour development following dietary inclusion of cypermethrin for up to 2 years in rats.

The outcome of a combined chronic toxicity and carcinogenicity study performed in the rat showed no evidence of carcinogenicity. Although this older study was not completely performed in compliance with the guidelines, the results can be regarded as adequate.

Non-rodent

A waiver argument for the second non-rodent species carcinogenicity was submitted by the applicant and was considered acceptable by the RMS.

The results of in-vitro and in-vivo genotoxicity studies all indicate that the test material is non-genotoxic. The studies covered would allow for both the assessment of clastogenicity and mutagenicity. As a result there is no evidence to suggest that the material is a genotoxic carcinogen.

The results of the combined chronic toxicity and carcinogenicity study in the rat shows no evidence of carcinogenicity. This result may also be supported by a lack of preneoplastic changes evident in a subchronic study in rat (see section 3.5: [redacted]). The primary site of toxic effect is the neuronal system. Toxicity does not seem to affect the proportion of neuronal type tumours.

For these reasons, and in order to minimise animal testing, further testing for carcinogenicity is not considered necessary for cypermethrin. It has been acknowledged that the data for the rat carcinogenicity study was not as complete as would be expected from similar studies conducted at this present time. However this does not detract from the quality of the results that were achieved from the study, which was also reviewed and accepted under Directive 91/414/EC.

Mechanism of action and supporting data (data from open literature)

[redacted] used the endpoint marker, glutathione S-transferase P (GST-P) positive liver cell focus larger than 0.2 mm in diameter as indicator of neoplastic development and for the evaluation of the carcinogenicity of cypermethrin. Cypermethrin was negative in this assay: Cypermethrin failed to induce the values of GST-P positive foci.

[redacted] confirmed that cypermethrin failed to induce the values of GST-P positive foci. Nevertheless, [redacted] reported that cypermethrin induced an increase in the number of foci per liver and in the percent tissue occupied by gamma-glutamyltranspeptidase (GGT) -positive foci in initiated rats.

According to the medium-term bioassays ([redacted]), cypermethrin at doses of 800 ppm (48 mg/Kg bw/d) did not enhance the development of GST-P positive foci in the liver.

[redacted] studied the carcinogenic and cocarcinogenic potential of the biocidal product ‘Cypermethrin 10% EG’ (Bilsyp) on the mouse skin. The results revealed that Cypermethrin 10% EG

possesses a carcinogenic as well as tumour initiating and promoting potential in both the sexes of Swiss mice in a long term in vivo carcinogenicity assay on mouse skin. However, there are substantial concerns relating to the substance tested, the actual dose of cypermethrin tested; a clear discrepancy in lethality of doses tested compared to GLP studies of cypermethrin, and inconsistency of tumour incidence compared to guideline dietary carcinogenicity studies where dermal contamination is likely to occur at levels that are probably comparable. A further possible uncertainty relates to accuracy of histological diagnosis, hypothesised from the absence of tumour progression. In conclusion, the reliability of the study is not high enough to use it to make decisions about the dermal carcinogenicity of cypermethrin.

Review of the [redacted] paper suggests the following points should be considered:

The mouse dermal carcinogenicity model is perceived as a useful study of carcinogenic potential, although no formal OECD guideline exists. [redacted] is noted to use the Swiss albino mouse, a readily-available but comparatively insensitive model compared to the SENCAR or genetically modified Tg.AC models which have been specifically developed for the purpose.

The Shukla et al. study carries no statement of GLP compliance. The test substance is described as: "Cypermethrin 10% EG (Bilsyp)". Thus it contains 10% cypermethrin, hence 90% of the material tested is not cypermethrin. Controls were either untreated, or treated with acetone; there was no control using a "formulation blank". Since 90% of the tested material was not cypermethrin, it is inappropriate to attribute the findings to cypermethrin. Further, the publication does not specify that dosage was corrected for 10% purity; so the doses stated may exaggerate exposures to cypermethrin itself by 10-fold.

There was no control group showing the incidence of tumours that might result from 32 weeks of treatment with TPA in isolation.

Survival of mice treated with the cypermethrin formulation in this study appears poor. Assuming (from the methods section) an initial group size of 20 mice per sex, the following survival is derived:

Survival in mice treated with cypermethrin

Treatment group	Phase of study (Duration)	
"Complete" carcinogenicity (52 weeks)		
Males		Females
Untreated control	19/20	20/20
B-a-P (5ug/mouse)	16/20	17/20
Cypermethrin (5 mg/Kg bw, 3x/week)	15/20	16/20
Cypermethrin (10 mg/Kg bw, 3x/week)	11/20	16/20
Cypermethrin (25 mg/Kg bw, 3x/week)	11/20	12/20
Acetone		
Initiating potential (32 weeks)		
Untreated control	19/20	20/20
DMBA + TPA	16/20	17/20
Cypermethrin (10 mg/Kg bw, single dose) + TPA	12/20	14/20
Cypermethrin (10 mg/Kg bw, 9 doses) + TPA	9/20	13/20
Cypermethrin (10 mg/Kg bw) + acetone	18/20	17/20

Acetone	19/20	19/20
Promoting potential (32 weeks)		
Untreated control	19/20	20/20
DMBA + TPA	16/20	17/20
DMBA + cypermethrin (10 mg/Kg bw, 3x/ week)	10/20	13/30
Acetone + cypermethrin (10 mg/Kg bw, 3x/week)	16/20	18/20
DMBA + Acetone	19/20	19/20
BaP: benz-a-pyrene; DMBA – dimethylbenzanthracene; TPA – tetradecanoylphorbol acetate		

Mortality appears strongly associated with application of cypermethrin in all three phases of the study. Considering the range of doses tested, or the multiplicity of doses tested, there is no good correlation of cypermethrin dose with mortality; a single dose of 10 mg/Kg was associated with as great a mortality (over 52 weeks) as 10 mg/Kg bw applied 3 times/week for 32 weeks. The time course of mortality is not reported. The apparent toxicity of cypermethrin in this study is remarkable, since from the CAR the dermal LD50 in rats is >2000 mg/Kg bw, and the dermal NOAEL (15-day rabbit study) is 20 mg/Kg bw/day. From GLP studies, no cypermethrin-related mortality would be expected from dermal application of doses up to 25 mg/Kg bw/day. This would be particularly the case if doses of "Bilsyp" were not corrected for cypermethrin content. Survival may have been affected by oral ingestion of the test substance. [redacted] does not include description of any procedure to cover the test sites or prevent ingestion of the test substance. Even so, the oral LD50 of cypermethrin in mice is listed in the CAR as >2011mg/Kg bw, and in a non-polar solvent, the LD50 (rat) is 250 mg/Kg bw, providing equally little reason to expect cypermethrin-related mortality.

It is noted that mortality in untreated control groups in all three phases of the study was identical (a single male in each case), raising the possibility that the untreated control was a single group common to all three phases. If this were to be the case, it is unclear if the single control group might have been of 32 or 52-week duration; if of only 32 weeks, control tumour incidence compared to groups used for 52 week would be under-reported. The historical incidence of tumours in relevant negative and positive controls is not reported, and would be useful for interpretation of the number of malignancies seen with BaP, or of total tumour count associated with TPA in isolation.

The test species/strain of animal used, the Swiss albino mouse, is a standard but not sensitive strain of mouse. An increase in tumour incidence was however seen with a dose level as low as 5 mg/Kg bw, 3x week over 52 weeks; if no correction for purity was made, the "Bilsyp" applied at this dose would be equivalent to as low as 0.5 mg/Kg bw/day cypermethrin. By comparison, alpha-cypermethrin was tested for carcinogenicity in groups of 52 CD-1 mice (CD-1 is a variant of the Swiss albino) at dietary concentrations of up to 300 ppm for 78 weeks (a dietary intake of 35 mg/Kg bw/day); no increase in tumour incidence was detected. Given that mice feed using their forepaws, then this study must represent a substantial exposure of skin (at least of the forepaws) to alpha-cypermethrin several times per day each day for 78 weeks; assuming dermal exposure as only 5% of oral intake then dermal exposures would be very close to those tested by [redacted]; but using a 2.5-fold greater group size, and a significantly longer exposure period (the increased duration also being significant for tumour expression). Given that similar strains of mouse were used in each study, it is clear there is a major discrepancy between the results which cannot be obviously reconciled.

The mouse skin model is specified as a test of the two-stage carcinogenesis process (initiation and promotion). There is however a third facet of the process (progression). The skin tumours associated

with cypermethrin exposure in [redacted] (approximately 240 in Document EWC ID:1005366.uk0 A0T0 0111 0002 Page 3 of 3

number) were all benign, and it would seem ("very few tumours were found malignant in BaP exposed animals") only a small fraction even of those resulting from BaP induction progressed to malignancy. This failure to progress to malignancy is curious, and historical control data from both positive and negative controls would be helpful in this respect. A suspicion can be raised on histological diagnosis (although diagnostic criteria are clearly stated) as to whether the transition from hyperplasia to metaplasia to neoplasia has been correctly assessed. It is however profoundly unlikely that all tumours attributed to cypermethrin were misdiagnosed, sufficient to invalidate the results of the study.

Summary of carcinogenicity: (from Doc. III-A 6.5/6.7)

Rat: Cypermethrin was tested in a combined chronic toxicity / carcinogenicity study. Overall, no effect on the number and type of tumours of the cypermethrin treatment (0.05, 0.5, 5, 50 mg/Kg bw/d, orally) was found in the Wistar rat.

Mouse: No test reports received from the applicant. The open literature revealed that the biocidal product 'Cypermethrin 10% EG' possesses a carcinogenic as well as tumour initiating and promoting potential on the mouse skin. However, the reliability of the study is not high enough to use it to make decisions about the dermal carcinogenicity of cypermethrin.

Classification/Labelling of the active substance 'cypermethrin' for carcinogenicity according to the criteria in Directive 67/548/EEC: **None**

Classification/Labelling of the active substance 'cypermethrin' for carcinogenicity according to the criteria in CLP-Regulation (EC) No 1272/2008: **None**

3.8 REPRODUCTIVE TOXICITY

3.8.1 Teratogenicity

Table 3.8.1.: Teratogenicity of cypermethrin

Route of exposure	Test type Method Guideline	TS	Species Strain Sex no/group	Exposure Period	Doses	Critical effects 1) dams 2) fetuses	NO(A)EL maternal toxicity	NO(A)EL Teratogenicity Embryotoxicity	Reference
Gavage	Method B of directive 87/302/EEC	Cypermethrin, WL43467, 98.5%	CD rat Females 25 / group	Day 6 – 15 of gestation	0, 17.5, 35, 70 mg/Kg bw vehicle: corn oil	1) Transient neurological disturbances at 70 mg/Kg bw/d Retardation in maternal bw gain at 35 and 70 Kg bw/d 2) Litter responses unaffected. No foetal abnormalities observed related to treatment.	17.5 mg/Kg bw	> 70 mg/Kg bw No embryotoxicity or teratotoxicity associated.	[redacted]
Gavage	Method B.31 of directive 87/302/EEC	Cypermethrin, WL43467, batch OCR/30 ST 76/001, 97.5%	New Zealand white rabbit Females control: 16 ♀ 20 mg/Kg bw: 22 ♀ 50 mg/Kg bw: 16 ♀ 120 mg/Kg bw: 17 ♀	Day 6 – 18 of gestation	0, 20, 50, 120 mg/Kg bw vehicle: corn oil	1) General condition unaffected. Body weight: trend towards reduction, but not stat. sign. No macroscopic changes observed at necropsy. 2) Litter responses unaffected. No foetal abnormalities observed related to treatment.	120 mg/Kg bw	> 120 mg/Kg bw	[redacted]

Gavage	No guideline study	Cypermethrin, NRDC 149, technical grade, 62.8:37.2 *trans*:*cis*, 92.4% purity	Wistar rat Females 5/group	Day 7-16 of gestation	0, 50 mg/Kg bw/d vehicle: corn oil	1) No evidence of toxicity (food and water consumption, mortality, weight gain, pregnancy). No changes in peripheral blood or spleen cytotoxic function during postnatal period. 2) Unaffected litter responses and postnatal development. But: ↑ peripheral blood lymphocytes, ↑bone marrow cells, ↓spleen cells, ↓thymocytes from d15 (53%) to d90 (15%) after birth. <u>Thymocytes</u> Thymocyte subsets affected: DN, SP, CD4, CD8; DN and SP recovered. Thymocyte distribution: ↑immature cells on d90. Thymocyte display: Impaired ability to proliferate in response to ≠ conc ConcA and human recombinant IL-2; Impaired ability to produce and/or release IL-2 in response to mitogens. <u>Peripheral blood lympocytes:</u> ↑$CD5^+$, $CD4^+$, $CD8^+$ Tcells, ↑Con A- and human rIL-2-induced lymphocyte proliferation ↑peripheral blood NK and ADCC activities from d60-d120 and ↑% $NKP\text{-}RP1^+$ cells (activation NK cell cytotoxic function) <u>Spleen cells:</u> ↓ $CD5^+$, $CD4^+$, $CD8^+$ Tcells, ↓ Con A- and human rIL-2-induced lymphocyte proliferation ↓spleen NK and ADCC activities from d30-d120 <u>Plasma catecholamines:</u> ↑ Adrenaline, Noradrenaline, peak levels at d60 after birth	[redacted]
Gavage	No guideline study	Cypermethrin, NRDC 149, technical grade, 62.8:37.2 *trans*:*cis*, 92.4% purity	Wistar rat Pups 10/Litter	d10-16 after birth	0, 1 mg/Kg bw/d vehicle: corn oil	Pups tested at 21d, 30d, 60d, 90d after birth: Bw, kidney weight, kidney:bw ratio not altered. No signs of poisoning or gross behavioural abnormalities Renal dopamine receptors: d21: affinity (Kd) and density (Bmax value) of D1- and D2-like receptors not altered. Older animals: ↓affinity, ↑density of dopamine D1-like receptors; no affected affinity but ↓density D2-like receptors.	[redacted]

Gavage	No guideline study	Cypermethrin, NRDC 149, technical grade, 62.8:37.2 *trans:cis*, 92.4% purity	Wistar rat Male pups 10/group	D8-15 after birth	0, 1.49 mg/Kg bw/d vehicle: corn oil	No signs of poisoning or gross behavioural abnormalities. No differences in body weight. On day 35 of age: Open field studies: ↑spontaneous locomotor activity and rearing episodes, ↑center entries and time spent in the center Striatum: ↓ dopamine levels, ↑homovanillic acid levels, ↑carbonyl group formation, no lipid peroxidation, fluidity at different depths of plasma membrane not changed Blood: ↓glutathione peroxidase, superoxide dismutase unchanged, carbonyl group formation unaltered, lipid peroxidation occurrence, fluidity at different depths of plasma membrane not changed, ↓superoxide anion production.	[redacted]

Cypermethrin was examined for prenatal toxicity in rats and rabbits.

CD rats were administered cypermethrin 1% in corn oil by gavage at doses of 0, 17.5, 35, and 70 mg/Kg bw/d on days 6-15 p.c. ([redacted]). In the top dose (70 mg/Kg bw/d) and mid dose (35 mg/Kg bw/d) groups, body weight gain of the dams was reduced during the treatment period and post-treatment period (only top dose). In addition, half of the female animals of the top dose group displayed transient neurological disturbances ranging from slight splaying of the hind legs whilst walking to severe splaying of all limbs, involuntary jaw movements, convulsive spasms and hypersensitivity to noise. No adverse effects were noted in dams treated with 17.5 mg/Kg bw/d. Examination of the dams at necropsy (d21 of gestation) revealed no macroscopic changes that could be related to treatment with cypermethrin. Litter responses were unaffected by treatment of the dams. Examination of the foetuses at necropsy revealed a small number of abnormalities in all groups including the control (type and incidences previously been found to occur spontaneously in CD rats). Nevertheless, no foetal abnormalities were observed which could be attributed to treatment with cypermethrin at any dose level. In conclusion, there was no embryotoxicity or teratogenicity associated with the oral administration of cypermethrin in this study. The following NOAELs were achieved: NOAEL maternal: 17.5 mg/Kg bw/d; NOAEL developmental: >70 mg/Kg bw/d.

A study performed with rabbits, which were administrated cypermethrin in corn oil (0, 20, 50, 120 mg/Kg bw/d) by gavage from day 7 to day 19 post-insemination (p.i) ([redacted]), revealed no treatment related changes on maternal body weight, food intake, general condition, or necropsy findings. Some inter-group variations were recorded in maternal bw gain, but no adverse effects or treatment related trends were found. All dams successfully carried their young to term with the exception of 2 dams of the 20 mg/Kg bw group and 1 dam of the 120 mg/Kg bw group which aborted during the post-treatment phase but none were found to be treatment related. The number of implantations, viable young and resorptions, pre- and post-implantation losses, foetal and placental weights were unaffected. Litter responses were unaffected by treatment of the dams. Examination of the foetuses at necropsy revealed a small number of abnormalities in all groups including the control (type and incidences previously found to occur spontaneously in this strain of rabbit). Nevertheless, no foetal abnormalities were observed which could be attributed to treatment with cypermethrin at any dose level. In conclusion, there was no maternal, embryotoxicity or teratogenicity associated with the

oral administration of cypermethrin up to 120 mg/Kg bw/d in this study. The following NOAELs were achieved: NOAEL maternal: 120 mg/Kg bw/d; NOAEL developmental: >120 mg/Kg bw/d.

In conclusion and with regard to these studies, oral administration of cypermethrin to pregnant rats or rabbits during organogenesis at dosages up to 120 mg/Kg bw/d was without adverse effects upon the progress and outcome of pregancy.

The open literature

[redacted] showed that prenatal exposure of rats to cypermethrin resulted in an increased number of total peripheral blood lymphocytes and bone marrow cells, and decreased spleen and thymus cells. In parallel, there was an increase in natural and antibody-dependent cell cytotoxicity (ADCC) and natural killer (NK) cell numbers in the peripheral blood, whereas NK cell functions were reduced in the spleen. Additionally, the development of T-cells was affected as was shown by the marked thymocyte depletion and altered distribution of the thymocyte subsets which was accompanied by a decreased mitogen-induced T cell proliferation and IL-2 production ([redacted]). In [redacted] it was shown that the effect on the T cell distribution and functions in the peripheral blood and spleen was correlated with the increased noradrenaline plasma level. It was suggested that cypermethrin may affect lymphocyte migration and function via catecholamine release.

The effects of neonatal cypermethrin administration in rats (d10-d16 after birth) on the postnatal development of the renal dopamine receptors was investigated by [redacted] with radioligand binding assay techniques. Dopamine D1- and D2-like receptors were assayed in frozen kidney sections of 21d, 30d, 60d, and 90d old rats. Treatment was without effect on the affinity and density of dopamine D1- and D2-like receptors of 21day-old rats. In older groups, treatment reduced the affinity and increased the density of dopamine D1-like receptors whereas it was without effect on the affinity of dopamine D2-like receptors and decreased their density. It was concluded that cypermethrin impaired the expression of renal dopamine D1- and D2-like receptors at a dose (1 mg/Kg bw/d) at which no generalised toxicity was reported.

The neurological effects of cypermethrin administration in developing rats (d8-d15 after birth) were further investigated by [redacted]. No difference was observed in behavioural activities in male pups at weaning (d21). However, at d35 neonatal cypermethrin exposure resulted in increased locomotion activity and rearing episodes, indicating a dopaminergic impairment. This was confirmed by the lower dopamine and higher homovanillic acid levels in the striatum. In addition, a reduction of blood glutathione peroxidase content was measured, while no change in blood superoxide dismutase was observed. Carbonyl group formation increased in striatum, but not in erythrocytes. Lipid peroxidation occurred in erythrocytes, but not in striatum. No changes in fluidity at different depths of

plasma mambrane were measured in striatum or erythrocytes. The activation of monocyte NADPH oxidase by phorbol esters showed that superoxide anion production was reduced. It was suggested that neonatal exposure to cypermethrin resulted in long-lasting effects such as changes in open-field behaviour, striatal monoamine level, and increased oxidative stress.

3.8.2 Fertility

Table 38.2.: Fertility effects of cypermethrin

Route of exposure	Test type Method Guideline	TS	Species Strain Sex no/group	Exposure Period	Doses	Critical effect	NO(A)EL Parental	NO(A)EL F1	NO(A)EL F2	Reference
Oral feed	Three generation study / Comparable to but deviating from Method B.35 of directive 87/302/EEC	Cypermethrin, WL43467, batch 30, 98%	Wistar rats m/f 30 /sex / group	Premating (m & f): F0: 35 d (5 weeks)	0, 10, 100, 500 ppm (0, 1, 10,50 mg/Kg bw/d)	Adults: 50 mg/Kg bw: ↓bw and food intake at F0, F1, F2. Effects greater for females. Fertility, gestation, viability, lactation indices not altered. Litters: 50 mg/Kg bw: ↓litter size and weight and pup weight F0	m + f 10 mg/Kg bw/d (100 ppm)	10 mg/Kg bw/d (100 ppm)	10 mg/Kg bw/d (100 ppm)	[redacted]
							NOAEL parental: 10 mg/Kg bw/d NOAEL reproductive: 50 mg/Kg bw/d NOAEL developmental: 10 mg/Kg bw/d			

Oral tap water	No guidline study	Cyper methri n 10	Sprague-Dawley rats Male 8/group	Premating: 12 weeks, daily	0, 8571, 17143, 34286 ppm 0, 13.15, 18.93, 39.66 mg/rat/d (≈ 0, 40, 60, 120 mg/Kg bw/d)	Adult male: ↓bw gain for all 3 doses Fertility male: All doses: ↑total number of resorptions per group, ↑females with resorptions, ↓number of viable foetuses; 18.93, 39.66 mg/rat/d:↓pregnant females 39.66 mg/rat/d: ↓number of implantation sites All doses: ↑preputial gland weight, ↓epididymal and testicular sperm counts, daily sperm production, testes infiltrated with congested blood vessels with hemorrhage and accumulation of connective tissue surrounding the seminiferous tubules containing a large number of immature spermatids; 18.93, 39.66 mg/rat/d: ↑ testes weight, ↑ seminal vesicles weight, ↓perimeter and number of cell layers of seminiforous tubules; 39.66 mg/rat/d: ↓ serum testosterone, FSH, LH (hormone levels not measured for lower conc);	LOAEL sytemic and fertility: 40 mg/Kg bw/d	[redacted]
Oral	No guideline	Cypermethrin 25% EC	Rabbit New Zealand White Male, n=6	12 weeks	0, 24 mg/Kg bw every other day	*↓ 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.* Induction of free radicals in plasma, liver, brain, testes ↓GST (liver, brain, testes), AST and ALT (liver, testes), AP (liver) ↑GST, AST, ALT, AP (plasma) AChE: not changed in plamsa and brain ↓ rel. testes and epididymis weight Semen quality: ↓ejaculate volume, sperm concentration, total sperm output, sperm motility, total motile sperm per ejaculate, packed sperm volume, semen initial fructose Plasma testosterone level↓ ↑abnormal and dead sperms, initial pH (abnormal: coiled tail, tapering, small head)	LOAEL: 24 mg/Kg bw every other day	[redacted]

Oral	No guideline	Cypermethrin, technical grade, 75:25 *trans:cis*, 94% purity	ICR CD-1 mice	Premating (m&f): F0: 5d/week for 4 weeks	0, 2.5, 5, 10 mg/Kg bw/d vehicle: corn oil	Adults: 10 mg/Kg bw/d: Clinical signs including salivation, hyperactivity, tremors (m&f, females most affected); ↓ bw (gain) during treatment or gestation and lactation (f); ↓ pregnant females Offspring: 10 mg/Kg bw/d: Litters: ↓live pups and ↑dead pups (per litter), ↓pup weight gain Physical parameters: delay in the development of pinna detachment (47%), down appearance (64%), eye opening (39%); Altered development of reflexes (self-righting, negative geotaxis, cliff avoidance); Altered swimming ability (direction , head angle); Altered open-field activity and social interaction	NOAEL parental = 5 mg/Kg bw/d NOAEL developme nt = 5 mg/Kg bw/d	[redacted]

A 3-generation study, in the Wistar rat via the feed, was conducted by [redacted]. Rats received in the diet for 5 weeks, cypermethrin at doses of 0, 1, 10, 50 mg/Kg bw/d, each group comprised 34 females. Males and females from each treatment group were selected at random and caged together for mating. Two litters were produced from each pair for three successive generations. The second litter of each generation was weaned, fed the appropriate diet and mated to produce 2 litters ;F0A and F0B. Dietary exposure to the test material was continuous for all generations from F0 prior to mating through to the weaning of the F2B generations. Within this study, a depression in body weight and food intake was reported at intervals at the highest dose (500 ppm, 50 mg/Kg bw) for the F0/F1/F2 animals, these effects being more pronounced in females. Fertility, gestation, viability, and lactation indices were found similar for treated and control animals within each generation. At the highest dose (500 ppm, 50 mg/Kg bw) litter size and weights of F0A was reduced and mean pup weight were lower for F0B female pups and pups of both sexes and F2B male pups. No differences in mean pup weights were recorded for the F1A, F1B, and F2A litters at any dose level. Furthermore, there was no evidence of any neurotoxic responses in the peripheral or central nervous system following treatment of successive generations. Thus the following NOAELs were achieved for cypermethrin: NOAEL parental: 10 mg/Kg bw/d (100 ppm); NOAEL reproduction: 50 mg/Kg bw/d (500 ppm); NOAEL developmental: 10 mg/Kg bw/d (100 ppm).

Adult data

Endpoint/dose	0		1 mg/Kg bw/d		10 mg/Kg bw/d		50 mg/Kg bw/d	
Adult F0	♂	♀	♂	♀	♂	♀	♂	♀
Food intake			↓ 4% w3,4,5		↓ 4% w3,4		↓ 7% w3,7	↓ 6-7% w3,4,5,6,7
BW							↓	↓ 4-5%
Adult F1, F2								
Food intake			↓ 8% F1 w3					↓ w4 to 7 F1 6-8% ↓ w5,7 F2 11-16%
BW							↓ F1 4-5%	↓ F1 4-7% F2 5-6%

Litter data

Endpoint/dose	0	1 mg/Kg bw/d	10 mg/Kg bw/d	50 mg/Kg bw/d
Litter survival				↓ F0A d0,7,21
Litter size				↓ F0A d7to21
# ♀ pups /Litter		↑ F0B d1,21	↑ F0B d1,21	↓ F0A d1,21
Mean litter weight		↑ F0B d7		↓ F0A d4,14,21
Mean puppy weight		↑ F0A d14,21 ↓ F2B 10%, d21, ♂	↓ F2B 11%, d21, ♂	↓ F0B 10%, d21 ↓ F2B d21, ♂ 9%, ♀ 6%

In conclusion, cypermethrin administration to rats via feed over 3 consecutive generations at dose levels of 1, 10, and 50 mg/Kg bw/d, resulted in no effect on fertility and gestation. Toxicity in parental animals was only observed with respect to body weight and food intake. The effects observed in the pups were secondary to maternal toxicity. Furthermore, no evidence was found of any neurotoxic responses in the peripheral or central nervous system.

$NOAEL_{parental}$ = 10 mg/Kg bw/d

$NOAEL_{reproduction}$ = 50 mg/Kg bw/d

$NOAEL_{developmental}$ = 10 mg/Kg bw/d

<u>The open literature</u>

The toxic potentials of cypermethin on reproductive and fertility parameters in the male rat were studied by [redacted]. A decrease in body weight gain was observed for all treatment doses, indicating general toxicity. However, no clinical signs of toxicity were observed at any dose level. Several reproductive parameters were adversely affected after the ingestion of cypermethrin 10. The pregnancy rate, number of implantation sites, and the number of viable foetuses were reduced in females impregnated by cypermethrin exposed males. In addition, the serum levels of testosterone, FSH, and LH were reduced in males ingesting 39.66 mg/rat/d. The decreased male fertility was explained by the fact that cypermethrin acted directly on the testes and influenced the androgen biosynthesis pathway. This was supported by the abnormalities seen on the histological sections of the testes. The weight of the testes, seminal vesicles, and preputial glands were increased, and the epididymal and testicular sperm counts were decreased in a dose-dependent manner. Nevertheless, adverse effects on fertility and reproduction were seen at dose levels that induced general toxicity as shown by the decreased body weight gain (nutritional status is known to affect the male rat reproductive system). As such, cypermethrin might have affected the animals indirectly rather than having any specific effect on the reproductive function. No NOAEL could be achieved in this study.

The toxic effect of cypermethrin on the semen quality and testosterone levels of the male rabbit were investigated by [redacted]. The treatment with 24 mg/Kg bw every other day over 12 weeks caused a decrease in body weight gain and relative liver, spleen, kidney, testes and epididymis weight (as also described in [redacted], [redacted]). Additionally, testosterone levels were decreased, which was attributed to the reduction in testes weight. The decline in semen quality was seen as partly attributed to the reduction in serum testosterone. The treatment with cypermethrin caused an increase in the percentage of abnormal and dead sperm, while semen initial fructose

decreased. Additionally, a decrease in sperm motility was caused. Nevertheless, the effect of cypermethrin on sperm quality may be due to the decrease in plasma testosterone and/or indirectly by the induced general toxicity.

[redacted] studied the effects of cypermethrin on the behaviour of F1-progeny following exposure of its parents before mating. In this study, performed in mice, the top-dose of 10 mg/Kg bw/d was maternally and developmentally toxic with decreases in litter size, pup survival, and pup growth. In F1 pups significant behavioural deviations were shown, identified by decreases in the performance of reflexes, swimming behaviour, locomotion frequencies in the open field, and reduction in the time spent in active social interaction. However, these behavioural effects cannot be attributed directly to the developmental neurotoxicity of cypermethrin on the F1-pups. These behavioural effects are most likely due to maternal toxicity and stunting of growth of the pups rather than the neurotoxicity of cypermethrin per se. In conclusion, the NOAEL obtained in this study for developmental effects of cypermethrin in mice was 5 mg/Kg bw/d.

Summary of reproductive and development studies: (from Doc. III-A 6.8.1 and 6.8.2)

The three-generation study involving administration of the substance in the diet of the rat showed that cypermethrin exerts no effect on the different reproduction parameters or on the survival of the offspring.

$NOAEL_{parental}$ = 10 mg/Kg bw; $NOAEL_{reproduction}$ = 50 mg/Kg bw; $NOAEL_{developmental}$ = 10 mg/Kg bw.

The teratogenicity studies involving oral administration of cypermethrin during organogenesis at dosages up to 70 mg/Kg bw/d in rats and up to 120 mg/Kg bw/d in rabbits were without adverse effects upon the progress and outcome of gestation.

According to the open literature, cypermethrin induced functional impairments at the neurotransmitter receptor levels in neonatal rats. However, since the multigeneration reproduction study in rats was without any indication of persistent effects in the offspring, which were also exposed to cypermethrin neonatally, it is suggested that receptor binding changes are not predicitive or causally related to the behavioural changes. Moreover, the most vulnerable phase for humans during the brain growth spurt is prenatal and not post-natal as in rodents. Therefore, exposure of the human foetus will be limited by maternal pharmacokinetics as well as maternal toxicity. The decreased male fertility seen in the rat and rabbit as demonstrated in the open literature appeared to be an indirect effect as it was caused at cypermethrin doses inducing clear general toxicity.

Based on the available data, it can be concluded that there is no evidence giving rise to concern for an additional risk for the newborn or young humans that should trigger further investigations.

Classification/Labelling of the active substance 'cypermethrin' for toxicity to reproduction according to the criteria in Directive 67/548/EEC: **None**

Classification/Labelling of the active substance 'cypermethrin' for toxicity to reproduction according to the criteria in CLP-Regulation (EC) No 1272/2008: **None**

3.9 NEUROTOXICITY

3.9.1 Neuropathological studies

Table 3.9.1.: Neuropathological studies with cypermethrin

Type of study	Species	Doses applied, Duration of exposure	Results	NO(A)EL	Reference
Screening study of reproduction/development toxicity OECD 421 Oral/gavage	Rat	Dose level: 0, 5, 25 and 50 mg/kg bw/day Vehicle: corn oil Amount of vehicle: 5 mL/kg Exposure: daily from 2 weeks prior to pairing and until day 4 post-partum for the fermales and until day before necropsy in week 8 for the males.	-Mortality: one male of the top dose group was killed in week 8 following clinical signs of impaired mobility, hypersensitivity and a convulsive episode -Mouth rubbing, salivation and paddling of the forelimbs in all the male groups (including the control group) and in all female groups. These effects occurred mainly immediately post-dose or 0.5h after treatment. Effects are dose related. -Increase occurrence of organ alteration (testes, epididymis, prostate, urinary bladder and pituitary) at dose 25 and 50 mg/kg bw/day -At the top dose, arched gait, ataxia, Straub tail and lethargy were observed, mainly 2 hours post-dose or more. -At 25 mg/kg bw/day, 3 total litter loss	LOAEL parental: 5 mg/kg bw/day LOAEL developmental: 25 mg/kg bw/day NOAEL developmental = 5 mg/kg bw/day	[redacted]
Study of developmental neurotoxicity OECD 426 Oral/gavage	Rat	Dose level: 0, 5, 15 and 25 mg/kg bw/day Vehicle: corn oil Amount of vehicle: 5 mL/kg Exposure: daily for P females from day 6 of gestation to day 21 post-partum / By the milk for F1, no direct dosing	-Mouth rubbing immediately post-dose in all groups (including control). Dose-dependant. -Paddling of the forelimbs in all groups (including control). Dose-dependant -salivation in all the treated group during lactation period. Dose-dependant Significant difference on learning and memory in the swimming maze for group 25 mg/kg bw/day	LOAEL parental: 5 mg/kg bw/day LOAEL developmental: 25 mg/kg bw/day	[redacted]

Delayed neurotoxicity/ Protocol partly in compliance with OECD 418 Oral/gavage	Hen (domestic laying) 6/group	0, 1 g/Kg bw Cypemethrin: WL 43467, batch 30, 98% purity Vehicle: DMSO Positive control: received pralidoxime chloride and atropine sulphate and a single dose of 0.5 ml/Kg bw Tri-0-tolyl phosphate (TOTP). Dosing regime: 5 daily oral doses of 1 g/Kg bw After 3 weeks: dosing regime repeated After another 3 weeks: Necropsy.	Cypermethrin group: No deaths and no signs of poisoning at any time. No histological lesions in the peripheral or central nervous system. (tested: signs of ataxia, ability to land without staggering when forced to fly; histopathology of brain and sciatic nerves; cervical, thoracic and lumbar cords, sciatic nerve, cerebellum and medulla oblongata were processed for histopathology)	1000 mg/Kg bw	[redacted]

Delayed neurotoxicity/ No guideline study Oral/gavage	Rat (Long-Evans) m/f 8/sex/ group	*FOB study:* 0, 20, 60, 120 mg/Kg bw/day Observations 1.5 and 3, 24 and 48h after dosing *Motor activity study:* 0, 20, 60, 100 mg/Kg bw/day Experiments 3, 24, 48h after dosing (The top dose was reduced due to lethality noted in the FOB study) Cypermethrin 97% in corn oil	**Body weight:** At the top dose bw-loss (♂ 7%, ♀ 9%) still evident 48h after dosing for both m/f. **Signs of toxicity:** FOB: 1 ♂, 6 ♀ died at top dose, Motor activity: 2 ♂ died at top dose. **Functional changes:** Two phases of toxicity evident: Salivation and increased removal activity (m) more evident at 1.5h. At 3 h these signs were subsiding and pronounced motor and sensory effects were apparent. **Motor activity:** Markedly depressed on dosing day. All doses significantly decreased activity in both m/f; high dose still effective at 24h. The lose dose was close to the ED50 value for theis measure: decreased activity by 46% (m) and 43% (f). No effects obtained at 48h. **Behavioral effects:** After administration rats displayed pawing and burrowing behavior even while in open field. Swollen muscles may have been a direct effect of cypermethrin or due to irritation produced by the excessive burrowing action. Spontaneous vocalisation. Behavior changes: increased sensitivity to external stimuli, splayed hindlimbs and abnormal locomotion, decrease of sensorimotor reactivity, with exception of increased click response. Neuromuscular changes: decreased grip strengths, gait changes and altered righting ability. Muscle tone was decreased and evidenced by splayed legs, increased landing foot spread and flattened posture. Behavioral effects were significant at both the middle and the top dose. **Other:** Cypermethrin produced hypothermia in both m/f.	20 mg/Kg bw	[redacted]

No guideline study Motor activity study Oral/gavage	Rat (Long-Evans) Males	1 single administration Dose range: 0.1-120 mg/Kg bw, 6 different doses Cypermethrin (*cis:trans*/48.7:51.3; 8 isomers mixture; 88% purity) in corn oil Control: corn oil Motor activity measured for 1 h at time of peak effects 1.5hrs after dosing Method: figure-eight maze	**Motor activity:** Dose dependent decrease in motor activity ED30: 10.7 mg/Kg bw Threshold dose: 4.26 mg/Kg bw Relative potency on motor activity compared to deltamethrin: 0.235		[redacted]
No guideline study Assessing EEG activity IP	Rat (Wistar) Males Sham control n=10 Vehicle control n=5 Cypermethrin n=9	IP, daily for 10 days Cypermethrin: 300 mg/Kg bw (91% purity, 49.9% *cis-trans* relation) Vehicle: 20% pluronic Electroencephalographic activity recorded daily for 30 min immediately after dosing (started 6 days prior to dosing, and during the 10 days of dosing) Evaluation of abnormal behavior	**1-2 days of dosing:** 1 animal presented high-voltage epileptic spikes **5 days of dosing:** 7 animals presented high-voltage epileptic spikes This is in contrast with the total absence of paroxystic activities in the control groups and in the period prior to dosing. **Toxic effects:** Evident throughout the 10 days Most animals died 2-4 hrs after the 10th dosing and all with an abnormal EEG activity bfore death. Signs before death: diarrhea, immobility, generalised tonic-clonic seizures, generalised fine tremor, conjunctivitis. At necropsy: enlarged liver.		[redacted]

The investigations made specifically for the end-point "Delayed Neurotoxicity" consist of an oral acute and a repeated dose neurotoxicity study with cypermethrin in the rat and the hen, respectively.

A functional observation battery (FOB) study was performed to assess the effects of cypermethrin on the neurological function and behavior in the **rat** (open literature: [redacted]). 8 Adult rats/sex/dose received cypermethrin (97%) in corn oil, by gavage, at 20, 60, or 120 mg/Kg bw and were observed 1.5 and 3 h, 24 and 48 h after dosing. Motor activity experiments were performed at 20, 60, and 100 mg/Kg bw after 3, 24, and 48 h after dosing. After cypermethrin administration, rats displayed pawing and burrowing behavior, spontaneous vocalisation, increased sensitivity to external stimuli, splayed hindlimbs and abnormal locomotion, decrease of sensorimotor reactivity with exception of increased click response. Neuromuscular changes were decreased: grip strengths, gait changes and altered righting ability. Muscle tone was decreased as evidenced by splayed legs, increased landing foot spread and flattened posture. Behavioral effects were significant at both the middle and top dose. In conclusion, behavior representing all of the functional domains assessed was affected, indicating the broad neurological activity of cypermethrin. Cypermethrin produced pronounced neuromuscular weakness and equilibrium changes, retropulsion, lateral head movements, alterations in responses to various stimuli and increased urination. NOAEL = 20 mg/Kg bw.

In a repeated dose delayed neurotoxicity study ([redacted]) cypermethrin, 1000 mg/Kg bw in DMSO, was administered orally to 6 adults **hens** on 5 consecutive days. This dosing

regime was repeated after 3 weeks, and after another 3 weeks the hens were sacrificed. Hens were tested for their ability to land without staggering when forced to fly. Histology of the brain and the sciatic nerves was performed and blocks from cervical, thoracal and lumbar cords, sciatic nerve, cerebellum and medulla oblongata were processed for histology. Cypermethrin administration caused no mortality or clinical signs of toxicity at any time. No histological lesions were found in the peripheral or central nervous system. In conclusion, the repeated oral administration of cypermethrin produced no immediate or delayed signs of intoxicitation, nor any histopathological lesions in the nervous system of the adult laying hen. NOAEL = 1000 mg/Kg bw.

Further open literature

The relative potency of cypermethrin for acute effects on motor function on rats was recently calculated by [redacted]. Motor function was measured using figure-8 mazes. The relative potency was calculated based on the computed ED30s. Deltamethrin, with an ED30 of 2.51 mg/Kg bw was chosen as the index chemical. It was shown that cypermethrin produced a dose-dependent decrease in motor activity. ED30 = 10.7 mg/Kg bw; Threshold dose = 4.26 mg/Kg bw; Relative potency = 0.235.

[redacted] evaluated the effects of repeated exposure to cypermethrin by means of assessing the electroencephalographic (EEG) activity in the rat. Cypermethrin was administered daily for 10 days in a 300 mg/Kg bw IP dose. EEG activity was evaluated daily for 30 min immediately after dosing. High-voltage epileptic-like activity became clearly manifested as the days of poisoning elapsed, and this was sometimes accompanied by behavioral anomalies, such as shakes, myoclones, absences with lack of response to sensorial stimulation, and generalised tonic-clonic seizures with postictal EEG depression. A progressive increase in the number of bursts of epileptic activity, the total duration of the abnormal EEG activity, and the number of animals presenting these paroxysms was observed.

Based on the available data provided in the original dossier, there were no evidence giving rise to concern for an additional risk for the newborn or young humans that should trigger further investigations. According to results available to a similar substance, the WG-IV-2016 concluded that the applicant should provide a DNT study on cypermethrin six months before approval date of the active substance.

The post-approval data for Cypermethrin PT18 was provided by the applicant, Arysta Life Science Benelux sprl, via R4BP3 on 29.11.2019, containing 2 developmental neurotoxicity (DNT) studies:

- [redacted] Cypermethrin: Oral (Gavage) Screening Study of Reproduction/Development Toxicity in the Rat. (OECD 421).
- [redacted] Cypermethrin: Oral (Gavage) Study of Developmental Neurotoxicity in the Rat (OECD 426).

Regarding the OECD 421 study, the test item was administered for two weeks prior to pairing, during the pairing period and until Day 4 post-partum for the females and until the day before necropsy in Week 8 for themales. A similar group of 10 males and 10 females were given the vehicle, corn oil, over the same period to act as controls.The following were assessed: clinical observation, body weight, food intake, mating, litter data, organ weights, gross pathology and microscopic pathology.
There were 4 group doses: 0, 5, 25 and 50 mg/kg bw/day.

During the first 4 weeks of the dosing period for the males given 50 mg/kg bw/day and during pre-pairing period of the females given 50 mg/kg bw/day gained slightly less weight than the controls. During gestation, 14 female body weight gain was similar in all groups but during the first four days of lactation, mean body weight gain of the females given 50 mg/kg bw/d was lower than that of the controls. Since the full set of gestational data shows the opposite tendency to the lactation data from day

1 to 4 and since the mean body weight of dams from all dose groups were within the normal range for this rat strain, it was agreed at WG III 2023, that the decrease of body weight gain during day 1 to 4 of the lactation should not be considered as an adverse effect of Cypermethrin.

One male given 50 mg/kg bw/d was killed in Week 8 of the study (after the pairing period) following clinical signs of impaired mobility, hypersensitivity and a convulsive episode. Macroscopic and microscopic examination did not reveal a cause of death. There were no other deaths during the study.

Mouth rubbing, salivation and paddling of the forelimbs were noted in all dose groups from immediately post-dose up to the end of the day. The number of animals affected and the duration of the reactions were dose-related. It was agreed at the WG III 2023, that these effects should be considered as adverse and cannot be attributed to a bad taste or a bad gavage procedure of cypermethrin.

Mating data and numbers of pups born and pup viability were unaffected by treatment. Three females in the group given 25 mg/kg bw/d and two females in the group of 50 mg/kg bw/d showed total litter loss. Pups in the group given 50 mg/kg bw/d were slightly lighter than controls between Days 1 and 4 post-partum.
There were no effects of treatment on median pre-coital time, pregnancy rate, mating index or on the male and female fertility or fecundity indices.
Among the females with live pups on Day 4 post-partum, there was no effect of treatment on the number of pups born, viability to Day 4 post-partum or on pup sex ratio. The pups in the group given 50 mg/kg bw/d were slightly lighter than control on Day 1 post-partum and gained slightly less weight than the controls between Days 1 and 4 post-partum. Pup necropsy data were unremarkable.
An increased occurrence of organ (testes, epididymis, prostate, urinary bladder, pituitary) alterations at the top dose – 50 mg/kg has been described. Occurrences are also seen at 25 mg/kg bw/d for testes, epididymis and urinary bladder. However there was no effect of treatment on the testis/epdidymis weights.

Based on the immediate effects post-dose, it was agreed at the WG-III-2023 to set the parental LOAEL at 5 mg/kg bw/day. Based on the 3 total litter loss, the developmental NOAEL was set at 5 mg/kg bw/day.

Regarding the DNT study (OECD 426) [redacted] the developmental neurotoxicity potential of Cypermethrin was investigated in groups of 24 mated female rats treated at dosage of 5, 15 or 25 mg/kg/day daily from Day 6 of gestation to Day 21 post-partum, inclusive. Similar group of 24 rats was given the vehicle, corn oil, over the same period to act as controls.
The females were allowed to litter and rear their offspring to weaning. Offspring were randomly selected from within litters on Day 4 post-partum and assigned to different subgroups for behavioural tests, brain weights and neuropathological examination. The evaluations continued beyond weaning (filial, F1 generation) until the offspring were approximately 70 days old.
No direct dosing of the pups was needed since cypermethrin is present in the milk (dosing performed in this study).

Mouth rubbing, paddling of the forelimbs and salivation were noted immediately post-dose in all groups; It was agreed at the WG III 2023, that these effects should be considered as adverse and cannot be attributed to a bad taste or a bad gavage procedure of cypermethrin. Therefore the LOAEL for parental toxicity was set at 5 mg/kg bw/day.

Pup numbers, viability and mean pup weight gain during the lactation period were unaffected by treatment. There was no effect of maternal treatment on the brain weights of the pups killed on Postnatal Day 22. Exposure to Cypermethrin was demonstrated and the levels detected were approximately

related to maternal dose. There were no deaths during the F1 generation phases of the study. Group mean body weight and food intake were unaffected by maternal treatment.

However, there were significant differences between group 1 and group 4 (25 mg/kg bw/day) on learning or memory in the swimming maze trials, but only in males. Therefore the developmental LOAEL was set at 25 mg/kg bw/day (agreed during WG-III-2023).

Since we do not have this test for dose 5 and 15 mg/kg bw/day, no NOAEL was set.

3.9.2 Biochemical and electrophysiological studies

Table 3.9.2.: Biochemical and electrophysiological studies with cypermethrin

Type of study	Species	Doses applied, Duration of exposure	Results	NO(A)EL	Reference
No guideline study Biochemical study Neuromuscular dysfunction: Inclined plane test Peripheral nerve damage: β-glucuronidase and β-galactosidase Oral/gavage	Rat (Wistar) 8/sex/group	0, 25, 50, 100, 150, 200 mg/Kg bw for 7 consecutive days Cypemethrin 10% in DMSO Control: DMSO Necropsy 3-4 weeks after start of the experiment: SPTN dissected from spinal cord	**Mortality:** 200 mg/Kg bw: 4 (m), 5 (f) 150 mg/Kg bw: 1 (m), 2 (f) 100 mg/Kg bw: 0 (m), 1 (f) **Bodyweight:** Dose-related reduction in bw gain for the highest dose groups (200, 150, 100 mg/Kg bw) **Clinical signs:** Salivation, ataxia, splayed hindlimb gait, hyper-excitability to auditory stimuli, tremor, choreoathetosis. Not dose-related but severity and duration showed a dose-related increase. **Inclined plane test:** Significant functional deficit: maximal at end of 7 d dosing regime, dose-dependent. **Biochemical changes:** Distal section of SPTN: Increased β-glucuronidase and β-galactosidase at 150 and 200 mg/Kg bw/d proximal section of SPTN: Increased β-glucuronidase at 150 and 200 mg/Kg bw/d		[redacted]
No guideline study Electro-physiological study *In vitro*	Frog sciatic nerves	cypermethrin (10^{-5}M) in Ringer solution for 15, 30, 45, and 60 min	**Compound action potential in the isolated frog sciatic nerve:** Decrease in conduction velocity and significant decrease in the amplitude after 30, 45, and 60 min of treatment **Histopathology of the sciatic nerve section:** General structure showed degeneration: many axons in one part of the nerve compared with the other parts, and the diameters enlarged. Endoneurium of the same axons thickened.Also degenerated axons observed in other part of the sciatic nerve and more connective tissue between them. In this area, the structure of the endoneurium was obscured.		[redacted]

No guideline study Electro-physiological study *In vitro*	Oocytes of frog (*Xenopus laevis*)	Cypermethrin (1R, *cis*, αS isomer) in DMSO 0.3% and ND-96 medium Nominal conc: 100 µM Experiments performed during the stable period of channel modification following 6 min of perfusion with cypermetrhin and 3 min of washing with ND-96.	*Tail current kinetics:* Tail current persistent and fit by a biexponential decay *Pyrethroid-modified current:* Activated slowly (τ_{act}>10 ms), did not detectably inactivate during a 40-ms depolaristation The rate of activation of modified channels during a depolarizing pulse was strongly correlated with the rate of tail current decay following repolarisation. Cypermethrin exhibited a significant increase in fractional modification of $Na_v1.8$ sodium channels upon repeated stimulation.		[redacted]
No guideline study Biochemical study *In vitro*	Rat brain synaptosomal membrane	Ripcord (Cypermethrin 100 g/L, and xylene and petrol mixture 820 g/L) conc. cypermethrin: 0, 1, 10, 25, 50, 100 µM exposure: 1 hour	*1 µM:* ↑Mg^{2+} activated ATPase, ↓ Na^+, K^+-ATPase *10-50µM:* ↑Mg^{2+} activated ATPase Na^+, K^+-ATPase first ↓, then ↑ *100µM:* Inhibited ATPase activities		[redacted]

In a subacute oral study in the rat with cypermethrin 10% administration 0, 25, 50, 100, 150, 200 mg/Kg bw/d for 7 consecutive days, the neuromuscular dysfunction was assessed by the inclined plane test and peripheral nerve damage was assessed by using the biochemical markers β-glucuronidase and β-galactosidase measured in nerve tissue homogenates ([redacted]). Mortality occurred from 100 mg/Kg bw/d onwards. A dose-related transient functional impairment was maximal at the end of the 7-day dosing regimen. However, 10 days after dosing, no differences from control could be detected anymore. 3-4 Weeks after the start of dosing, a significant increase in β-glucuronidase and β-galactosidase activity was found in the distal portion of the sciatic/posterior tibial nerves (SPTN), though only at near-lethal doses (150 and 200 mg/Kg bw/d). In conclusion, no direct correlation was found between the time-course of the neuromuscular dysfunction and the neurobiochemical changes. Two distinct types of neurotoxic effects were suggested for cypermethrin: 1° a short-term transient pharmacological effect due to effects on the nerve membranes and 2° at near-lethal dose levels a more chronic neurotoxic effect that results in (sparse) axonal nerve damage.

Further evidence for the neurotoxic effect of cypermethrin on the peripheral nerves has been afforded by electrophysiological studies in the frog (Short communication: [redacted]). The peripheral nerve effects were characterised by investigating the frog sciatic nerve in vitro using the extracellular recording technique. The nerve compound action potentials were recorded before and after cypermethrin treatment (10^{-5}M in Ringer solution) for 15, 30, 45, and 60 min. A decrease of the conduction velocity and amplitude of compound action potentials was observed after 30, 45, and 60 min of treatment which might be the result of a slight demyelination lesion. The neurotoxic effect of cypermethrin on peripheral nerves, was observed especially at the axons and the endoneurium and was highly correlated with exposure time.

[redacted] performed an electrophysiological study of the action of cypermethrin on the voltage sensitive rat $Na_v1.8$ sodium channels expressed in frog oocytes. Cypermethrin produced clearly detectable tail currents. Measuring of the pyrethroid-modified current during the first

depolarizing pulse, resulted in a clearly detectable resting modification of $Na_v1.8$ sodium channels with characteristic pyrethroid-induced tail currents that occurred following repolarization. The tail currents were found persistent and were fit by an biexponential decay. Channels activated slowly (τ_{act}>10 ms) and did not detectably inactivate during a 40-ms depolarisation. A strong correlation was found between the time constants of activation and tail current decay. This is consistent with the hypothesis that the kinetics of tail current decay reflect a slowing of deactivation rather than the rate of dissociation of the pyrethroid from the channel. Although $Na_v1.8$ sodium channels in mammals are restricted in distribution to peripheral sensory neurons and are therefore not likely to be target sites that mediate the systemic acute neurotoxicity of cypermethrin, $Na_v1.8$ sodium channels may be important targets for cypermethrin in the production of paresthesia following dermal exposure.

[redacted] studied the activity of the membrane-bound integral protein ATPase as a biomarker for the toxic effects of cypermethrin in isolated rat brain synaptosomal membranes. The activities of total ATPase and Mg^{2+} activated ATPase were studied by determining the release of inorganic phosphate from ATP. Cypermethrin caused a significant increase in Mg^{2+} activated ATPase and decrease in Na^+, K^+-ATPase already at 1µM. At 10-50 µM there was a signifcant increase in Mg^{2+} activated ATPase. However, cypermethrin did not show a clear dose-dependent effect on Na^+, K^+-ATPase, as decreases were followed by increases. All ATPase activities were significantly inhibited at 100 µM. It was suggested that ATPases are activated to take care of the elevated ion concentrations because (as according to [redacted]) both the opening and the closing of sodium channels are slowed down by pyrethroids, resulting in delayed and prolonged openings.

Additonally, it has also been described that cypermethrin has a noncompetitive inhibitory effect on the $GABA_A$ receptor-ionophore complex in rat brain preparations and the guinea-pig intestine ([redacted]; [redacted]).

Summary of neurotoxicity studies:

Repeated oral dosing of adult laying hens with 1000 mg/Kg cypermethrin produced no immediate or delayed signs of poisoning, nor any histopathological lesions in the nervous system. However, the hen sciatic nerve would not be suitable for studying pyrethroid-induced nerve damage, as – in contrast to organophosphates – birds are highly insensitive to pyrethroids ([redacted]). In contrast with hens, rats treated with a single dose of cypermethrin (60 mg/Kg bw) showed behavioral changes indicating a broad neurological activity of cypermethrin. The clinical signs observed are characteristic for the acute poisoning with a type II pyrethroid: choreoathetosis accompanied by salivation (CS syndrome). Also in the rat, it was observed that cypermethrin produces epileptic activity during repeated administration. The neurotoxic effect of cypermethrin on peripheral nerves (axons, endoneurium) was highly correlated with exposure time. Cypermethrin exerts its toxicity by opening the voltage-gated sodium channel slowly for extended times, leading to a prolonged sodium current in the target neurons. Furthermore, the decrease in the Na^+, K^+-ATPase pump activity is involved in the paroxysmal epileptic activity induced by cypermethrin. Cypermethrin also inhibits $GABA_A$ receptors.

In conclusion, cypermethrin has a neurotoxic potential in rats.

3.10 OTHER

3.10.1 Immunotoxicity

Table 3.10.: Immunotoxicity studies with cypermethrin

Type of study	Species	Doses applied, Duration of exposure	Results	NO(A)EL	Reference
Immunotoxicity Oral **Rabbit** Humoral immune response (immunisation with S. typhi, antibody titer measuring by tube-agglutination and complement-binding test) Cell-mediated immune response (Tuberculin skin test) **Rat** Humoral immune response (immunisation with ovalbumin, antibody titer measuring by the passive hemagglutination test) Cell-mediated immune response (counting of the number of autologous rosette-forming T-cells)	Rabbit, male Rat, CFY male	Cypermethrin: 93.5% purity, Ripcord Rabbit: 0, 75, 150, 300 mg/Kg bw/d for 6 weeks in gelatine capsules Control: corn oil Rat: 0, 6.25, 12.5, 25 mg/Kg bw/d for 6 or 12 weeks, mixed in the food	**Rabbit** Humoral immune response: 300 and 150 mg/Kg bw/d: significant dose-dependent decrease in the antibody titer from the 2nd week onwards Cell-mediated immune response: 300 and 150 mg/Kg bw/d: significant dose-dependent decrease in the diameters of skin redness commared with controls from the 2nd week onwards **Rat** Humoral immune response: 25 mg/Kg bw/d: significant decrease in anti-ovalbumin titer Cell-mediated immune response: 12.5 and 25 mg/Kg bw/d: significant decrease in the autologous rosette formation of T-lymphocytes	**Rabbit** 75 mg/Kg bw/d **Rat** 6.25 mg/Kg bw/d	[redacted]

Immunotoxicity Oral **Goat** Cell-mediated immune response (Chemical contact sensitisation of skin: sensitisation with DNFB, skin thickness measuring) Humoral immune response (Enumeration of haemolytic plaques of antibody-forming cells) **Mouse** Cell-mediated immune response (Chemical contact sensitisation of skin: sensitisation with DNFB, ear thickness measuring)	Goat Black Bengal Male 3/group Mouse Swiss Male 10/group	Cypermethrin: no more data Goat: 0, 41.6 mg/Kg bw/d for 30 days, drenched Control: distilled water Mouse: 0, 50 mg/Kg bw/d i.p. Control: saline	**Goat** Cell-mediated immune response (DNFB test): Erythema, swelling, vesiculation, scabbing, sloughing of the skin caused by DNFB, and increase in skin thickness: reaction less pronounced in cypermethrin treated group Humoral immune response (haemolytic plaque test): Number of antibody-forming cells decreased. Mean diameter of haemolytic plaques smaller Histopathology: White pulp of spleen showed a washed –out appearance, and smaller Malpighian corpuscles were observed. Mesenteric lymph nodes: depletion of lymphocytes **Mouse** Cell-mediated immune response: Erythema, oedema, visiculation, induration and scrabbing caused by the challenge of DNFB was less pronounced in the cypermethrin treated group. Histopathology: Depletion of lymphocytes in the Malpighian corpuscles of the spleen		[redacted]
Immunotoxicity Oral, gavage Humoral: mean serum haemagglutinin titres and haemolysin titres against sheep RBC on d90 Cellular: delayed skin hypersensitivity (skin reactivity to tuberculin, skin thickness) + toxicological and haematological examination	Rat (Wistar) Males 7/group	0, 5, 10, 20, 40 mg/Kg bw/d for 90 days Cypermethrin, technical grade Vehicle: ground nut oil	**Toxicological / haematological :** *20 mg/Kg bw/d* ↓spleen weight, ↑ adrenal weight *40 mg/Kg bw/d* ↓spleen weight, ↑ adrenal weight, leucopenia on d90 **Immuno function tests:** Cell-mediated: Dose-dependent decrease in delayed type hypersensitivity reaction on d61 post-treatment 20 mg/Kg bw/d: 24% DTH ↓ 400 mg/Kg bw/d: 27% DTH ↓ Humoral: No definite pattern in the humoral response	NOAEL immunotox: 10 mg/Kg bw/d	[redacted]

Immunotoxicity Oral, gavage IgM-PFC assay DTH assay (Footpad swelling assay) + toxicological and haematological examination	Rat (Wistar) Males 10/group	0, 11.1, 22.2, 55.4 mg/Kg bw/d for 28 days Cypermethrin, 98.2% purity, cis:trans/39.4:60.6 Vehicle: sunflower oil	**Toxicological :** No toxic clinical signs and no changes in the behaviour observed, nor macroscopic changes during necropsy. No effect on bw or relative organ weight. **Haematological:** 55.4, 22.2 mg/Kg bw: ↓WBC, Ht, MCV 11.1 mg/Kg bw: ↓Ht, MCV **Immuno function tests:** *Splenic plaque forming cell (PFC) assay:* No effect on the splenic PFC count *Delayed type hypersensitivity (DTH)- footpad swelling assay:* All doses ↓ DTH reaction 24h and 2 higher doses ↓ DTH reaction 48h after challenge, dose-dependent	NOAEL immunotox: 11.1 mg/Kg bw/d	[redacted]

The immunotoxic potential of cypermethrin has been demonstrated in several studies.

[redacted] studied the influence of cypermethrin on the humoral and cell-mediated immune response in the rabbit and the rat. Rabbits fed with 300, 150, and 75 mg /Kg bw/d for 6 weeks, showed a significant dose-dependent decrease (2 highest doses) in the tuberculin reaction and in the anti-*Salmonella typhimurium* antibody titre. Rats fed 6.25, 15.5, and 25 mg/Kg bw/d for 6 or 12 weeks showed a significant dose-dependent decrease (2 highest doses) in the anti-ovalbumin titre of blood sera measured in the passive hemagglutination test, and a decrease in the autologous rosette formation of splenic lymphocytes.

The immunosupressive effect of cypermethrin is also observed in mice and goats ([redacted]). Mice were i.p. injected with cypermethrin 50 mg/Kg bw/d for 26 days, and goats were drenched with cypermethrin 41.6 mg/Kg bw/d for 30 days. Cell-mediated immunity (CMI) was assessed by the 2,4-dinitro fluorobenzene (DNFB) skin sensitivity test. In both mice and goats, a significant depression in CMI was observed. Additionally, the humoral response of the goat was estimated by enumeration of the plaque-forming B-cells. The rate of plaque formation and the diameter of the plaques was significantly reduced.

In a short communication, [redacted] 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 leucopenia at 40 mg/Kg at d90, and a dose-dependent decrease in delayed type hypersensitivity (DTH) reaction was noticed on d61 post-treatment. Humoral response as evidenced by serum haemagglutinin and haemolysin titres did not show any definite pattern at d90. Nevertheless, a significant decrease in spleen weight and a significant increase in adrenal weight was observed at 40 mg/Kg bw.

The immunotoxic effect of cypermethrin (0, 11.1, 22.2, 55.4 mg/Kg bw/d) was investigated in a 28-day oral study in male Wistar rats ([redacted]). Humoral and cell-mediated immunity was

investigated by the splenic plaque forming cell (PFC) assay and delayed type hypersensitivity (DTH) reaction (footpad swelling assay). No changes in general toxicity were observed. However all doses decreased the mean cell volume (CMV) of the red blood cells (RBC) and the haematocrit (Ht). The 2 higher doses also reduced the white blood cell (WBC) count in the peripheral blood. Among the immune function assays, only a dose-dependent decrease in the DTH reaction was observed. This is in line with [redacted].

The latter 2 studies show contradictory results, with in respect to the humoral response, to the results obtained by [redacted] and by [redacted], but these can possibly be explained by the different end-points measured, species variation, and the different purity and *cis:trans* ratio of the cypermethrin used.

In summary, cypermethrin induces immunosuppression: both the humoral and cell-mediated immune response are impaired by cypermethrin.

3.10.2 Endocrine Disruption

Table 3.10.: Endocrine disrupting activity studies with cypermethrin

Type of study	Species	Doses applied, Duration of exposure	Results	NO(A)EL	Reference
Estrogenic or antiestrogenic activity *In vitro* ERα-mediated assays: Luciferase reporter gene assay (mammalian cell-based) Yeast two-hybrid assay (Nishikawa et al., 1999: induction of β-galactosidase activity) Competitive ligand-binding assay: competitive binding to hERα by fluorescence polarization method (Bolger et al., 1998)	HeLa cells Yeast strain Y190	Cypermethrin, >93% 100 nM-10 µM in DMSO (max. dose level determined on basis of chemical solubility) Positive controls included	Cypermethrin did not show a significant estrogenic or antiestrogenic activity in the luciferase reporter gene assay, the yeast two-hybrid assay, and the competitive ligand-binding assay. The expected dose-response with the 3 assays was confirmed with 17β-estradiol. Positive controls showed (anti)estrogenic activity. **No estrogenic or antiestrogenic activity by classic ER-mediated pathways *in vitro***		Saito et al., 2000
Agonistic or antagonistic activity *In vitro* PRα-mediated assays: Luciferase reporter gene assay (mammalian cell-based) Yeast two-hybrid assay (Nishikawa et al., 1999: induction of β-galactosidase activity) Whole cell binding assay (Eil et al., 1980) and Cell-free binding assay (Sarup et al., 1988)	T-47D cells Yeast strain Y190	Cypermethrin, >93% (max. dose level determined in a control assay by luciferase activity: 10µM) Positive controls included	Cypermethrin did not show any binding to the progesterone receptor in the receptor binding assays, nor agonistic or antagonistic effects in the luciferase reporter gene assay, the yeast two-hybrid assay at the max dose level of 10 µM. Positive controls showed agonistic and antagonistic activity. **No agonistic or antagonistic effect on the human progesterone receptor *in vitro***		Sumida et al., 2001
Estrogenic or antiestrogenic activity *In vitro* ERα-mediated assays: E-screen assay (modified Villalobos et al., 1995) ER competitive binding assay (modified Blair et al., 2000) pS2 expression assay	MCF-7 cells Rat uterine cytosol	Cypermethrin, > 90% Positive control included	Significant proliferation of MCF-7 cells at 10nM: effect = 1.46 (144 hrs exposre) Inhibition of the binding of [3H]estradiol to ER at high concentration (EC_{50} = 0.562 mM), blocked by addition of ICI 182,780. pS2 mRNA expression induced (6 hrs exposure) **ER-specific, agonistic response *in vitro***		Chen et al., 2002

Estrogenic or antiestrogenic activity *In vitro* ERα-mediated assays: E-screen assay (modified Soto et al., 1995) Competitive ER binding assay (modified Kim et al., 2001) Mechanism studies: pS2mRNA expression assay ER (ERα, ERβ) expression	MCF-7 BUS cells Rat uturi cytosol	Cypermethrin, 98% Positive control included	Cypermethrin did not induceMCF-7 BUS cells at any concentration tested and did not affect 17β-estradiol-induced cell proliferation. Cypermethrin did not compete with [3H]estradiol for binding to ER at any concentration. **No estrogenic or antiestrogenic activity by classic ER-mediated pathways *in vitro*** Positive control (17β-estradiol): Induced cell proliferation in a dose-dependent manner. Maximum proliferative activity at 10^{-10} M, proliferation rate 4.8 folds higher than control. 17β-estradiol effectively competed with [3H]estradiol for binding to ER, IC_{50} = 5 x 10^{-10} M		Kim et al., 2004
Cell proliferation Bioluminescence assay for ATP **Cytotoxicity** WST-1 test: determination of mitochondrial metabolic activity	MCF-7 cells	Ripcord: 100 g/L cypermethrin, with 820 g/L mixture of xylene and petrol. Concentrations: 1-100 µM Exposure for 7 days with and without co-exposure to 0.1nM oestradiol	Exposure to 0.1 µM cypermethrin with 0.1nM oestradiol increased the total ATP to 373% compared to control. Without oestradiol, the ATP content after 0.1µM cypermethrin exposure was 125% compared to control. Cypermetrhin caused a significant increase in ATP content at the 0.1-1µM conc. Cell toxicity of cypermethrin started at 10 µM. WST-1: no corresponding effect seen		Kakko et al., 2004
Aromatase activity Aromatase assay: Tritiated water assay (2h and 24h exposure) Cyp19 mRNA measurements Trans activation assay: Luciferase assay	JEC-3 cell line	Cypermethrin Positive control included	Altering steroidogenesis: Cypermethrin induces aromatase activity after 24h exposure from 3 µM on. Effect on cyp19 mRNA expression: cypermethrin: 1.4-fold, not stat. sign. compared with solvent control. No induction of luciferase activity in the JEG-3 RLN cells. (preliminary MTT test for cell viability after 24h: 10 µM no alterations) **Induction of aromatase activity by cypermethrin is not mediated by RARs in JEG-3 cells**		Laville et al., 2006
Androgenic or antiandrogenic activity Reporter gene assay (human AR mediated luciferase reporter gen)	Cv-1 African green monkey kidney cells	Cypermethrin, >99% 3-PBA Concentration: 100µM Positive controls included	Androgenic activity: none Antiandrogenic activity: RIC_{20} cypermethrin: 0.42 mM RIC_{20} 3-PBA: 1.21 mM Cell viability tested in MTT assay. No cytotoxic effect found in the tested concentrations up to 100µM. **Cypermethrin and its metabolite 3-PBA show antiandrogenic activity *in vitro*, but no androgenic activity.**		Sun et al., 2007

The possible estrogenic and antiestrogenic activity of cypermethrin was evaluated by Saito et al. (2000), using 3 *in vitro* assays (cell-based luciferase reporter gene, yeast two-hybrid, and competitive ligand-binding assay) with classic ligand-mediated activation mechanisms. According to Saito et al. (2000), the lack of significant effects at 100 nM-10 µM indicates that cypermethrin is not estrogenic or antiestrogenic by classic human estrogen receptor α (*h*ERα)-mediated pathways *in vitro*.

In addition, Sumida et al. (2001) reported that cypermethrin shows no binding to the human progesterone receptor (hPR), agonistic or antagonistic effects, using 3 *in vitro* assays (cell-based luciferase reporter gene, yeast two-hybrid, and binding assay).

The estrogenic activity of cypermethrin activity was also studied by Chen et al. (2002) using 3 *in vitro* assays (E-screen assay, estrogen receptor (ER) competitive binding assay, pS2 expression assay). Cypermethrin significantly induced MCF-7 cell proliferation. Additionally, a high concentration of cypermethrin inhibited the binding of [3H]estradiol to ER and induced pS2 mRNA expression. As such, cypermethrin was found to produce an ER-specific, agonist response.

In contrast with Chen et al. (2002), Kim et al. (2004) obtained negative results for cypermethrin in the E-screen assay and ER competitive binding assay. It is known that false positive and false negative responses have been found depending on the exact method used and origin of the MCF-7 cell line (and serum and passage number) used.

In another series of testing with the MCF-7 cell line, Kakko et al. (2004) demonstrated that cypermethrin enhanced the proliferative effect of 0.1 nM oestradiol, when applied at low concentrations (0.1-1 µM) together with oestradiol to MCF-7 cells. This was evidenced with the ATP test, but not with the WST-1 test. Cypermethrin was cytotoxic from 10 µM onwards.

Cypermethrin was screened for its ability to modulate aromatase activity in the human choriocarcinoma JEG-3 cell line by using the tritiated water assay, and further with the retinoic acid receptor (RAR) signalling pathway (Laville et al., 2006). Cypermethrin was found to induce aromatase activity after an exposure of 24h from a concentration of 3 µM onwards. However, no effect on the cyp19 mRNA expression could be detected, and no luciferase activity could be induced in the JEG-3-RLN cells. In conclusion, Laville et al. (2006) suggested that the induction of aromatase activity by cypermethrin is not mediated by RARs in JEG-3 cells.

As results from animal studies on the reproductive system showing that treatment with cypermethrin resulted in a significant decrease in male fertility in the rat ([redacted]) and in the rabbit ([redacted]) although at concentrations inducing general toxicity, cypermethrin and its metabolite 3-phenoxybenzoic acid (3-PBA) were tested for their ability to induce androgenic or antiandrogenic activity *in vitro* using the reporter gene assay in CV-1 cells (Sun et al., 2007). A weak antiandrogenic activity was demonstrated at the high concentration of 0.1 mM (not found cytotoxic in this study) for both cypermethrin and its metabolite 3-PBA. No androgenic activity could be demonstrated.

Conclusion on the endocrine disruption activity:

The estrogenic potential of cypermethrin cis:trans/40:60 based on ER-mediated mechanisms remains equivocal. Contradictory results were revealed in different studies. In summary, the estrogenic and antiandrogenic effect of cypermethrin cis:trans/40:60 (and pyrethroids in general) depend on the assays or cells used. Results indicate that data obtained with high concentrations (> 10 µM) should be interpreted carefully (solubility of test chemical, cell toxicity). Possibly, cypermethrin cis:trans/40:60 is

an estrogen-like chemical that might act through signalling pathways other than direct ER binding, and as such, might function as an endocrine modulator. However, at present no definite conclusions can be drawn.

In November 2016 , the criteria for identification of endocrine disrupters are still under discussion for the biocide regulation. The entry into force is foreseen for 2017.

Therefore, eCA suggest to to consider the available studies at the renewal stage of cypermethrin for PT8 or PT18.

3.11 HUMAN DATA

Medical surveillance on manufacturing plant personnel

Clinical neurological examinations and electrophysiological studies have been conducted on 23 volunteer subjects. These persons had been occupationally exposed to synthetic pyrethroids, including cypermethrin, over a period from less than a year up to five years, and had experienced the typical paresthesiae on more than one occasion. This abnormal facial sensation developed between thirty minutes and three hours after exposure and persisted for thirty minutes to eight hours. It is not clear from the publication whether the workers had worn masks and/or gloves. Electrophysiological studies were conducted on selected sensor and motor neurons in the legs and arms. This study failed to show any significant abnormality of either motor or sensory nerves (Le Quesne et al., 1980).

Clinical cases and poisoning incidents

Despite their extensive world-wide use, there are few reports of human pyrethroid poisoning. According to Bradberry et al. (2005), less than ten deaths have been reported from ingestion or following occupational exposure.

A fatal case of human poisoning has been reported by Vijvenberg and Van den Bercken (1990). A 45-year old man, who had accidently ingested more than 0.7g cypermethrin 10%, rapidly developed convulsions, passed into a coma, and died 3 hours later. The death of this man was ascribed to respiratory paralysis.

He et al. (1989) reviewed 573 cases of acute pyrethroid poisoning reported in the Chinese medical literature between 1983-1988. Forty-five cases of acute cypermethrin poisoning were detected (6 occupational, 39 accidental). Apart from the irritative symptoms of the skin and respiratory tract (or digestive tract in ingestive poisoning), acute pyrethroid poisoning was clinically characterised by abnormalities of nervous excitability. Initial symptoms were burning or itching sensation of the face or dizziness which usually developed at 4-6h after exposure. The skin symptoms could appear early after several minutes of spraying, followed by systemic symptoms as late as 48h after exposure. Symptoms and signs of acute poisoning including the following: Half of the occupationally exposed patients had abnormal facial sensations which could be exacerbated by sweating and washing with warm water. The systemic symptoms included dizziness, headache, nausea, anorexia, and fatigue. Weakness was found in 53.4% of the cases. Other symptoms included chest tightness, paresthesias, and in 11.9% of the cases palpitations, blurred vision, and increased sweating. Several patients showed low-grade fever and myopia. Infrequently, seizures, pulmonary oedema, dyspnea, and cyanosis occurred.

Lessenger (1992) reported 5 cases of poisoning by cypermethrin. After treating the air-conditioning system with cypermethrin, creating a fine vapour, employees were allowed to enter the treated building after 2 days. Already after 5 minutes the exposed employees experienced shortness of breath, dyspnea, wheezing, cough, congestion, nasal discharge, burning eyes, itching skin, nausea, and headaches. The employees could re-enter the building repeatedly and when they did, they experienced a return of their symptoms after turning on the air-conditioning system. Five employees presented for examination. Shortness of breath persisted for over 2 weeks, and sore throat and sinus infections were still persistent 7 months post-exposure in one patient (non-smoker). Three other patients without previous pulmonary problems (of which 2 smokers) developed significant pulmonary dysfunction (still complaining of cough, congestion, and wheezing) 7 months post-exposure.

Das and Parajuli (2006) recently reported a case of cypermethrin poisoning in Nepal. A 30-year old man was brought to the emergency department of the hospital with a history of vomiting, epigastric pain, lacrimation, sweating and salivation after the ingestion of 50 ml of "Super-Cyprin" having a concentration of 25% cypermethrin (12.5 g). General examination revealed red and swollen lips and buccal mucosa, but vital and systemic examination were unremarkable. No fasciculation or tremor occurred and the liver function, renal function, SpO_2, haemogram, serum electrolytes and glucose tests were normal. A symptomatic treatment was given including administration of activated charcoal, hyoscine butyl bromide for non-specific abdominal pain, and chlorpheniramine maleate for the increased salivation and red irritating eyes.

Observations on exposure of professional operators and the general population

He et al. (1988) reported the effects of pyrethroid exposure (including cypermethrin) in persons engaged in packaging pyrethroids. In this health survey conducted on 199 workers, burning sensations and tightness or numbness on the face appeared in two thirds of the subjects, and one third had sniffs and sneezes. Abnormal facial sensations, dizziness, fatigue, and miliary red papules on the skin were more evident in summer than in winter. Neither abnormalities in other organs or systems nor symptoms or signs of acute pyrethroid poisoning were found in interviews, clinical examinations, and laboratory tests.

Only few cases of contact dermatitis due to the exposure to cypermethrin have been reported. Wagner (1994) reported two cases. The first case was a 40 year old woman whose home was treated with cypermethrin. A spill occurred which contaminated the flooring of the bedroom and closet. The following day she developed a generalized urticarial eruption and on the second day post-application she was seen at the emergency because of increasing urticaria which had progressed to involve her eyelids. This case of contact allergic dermatitis, was confirmed with a patchtest with the formulated product. The second case was an applicator treating an area with a combination of cypermethrin and cyfluthrin. Apart from the parasthesias of the exposed skin, this worker also developed an urticarial reaction.

In a study investigating the role of pyrethroids with respect to irritation and their sensitisation potential, Lisi (1992) tested 7 pyrethroids in 230 subjects (54 patients with contact dermatitis, 176 with non-allergic skin disorders, 16 atopics) from different areas (males and females; agricultural workers, ex-agricultural workers, others) to establish the optimal test concentrations in the patch test and the frequencies of irritant and allergic reactions. Cypermethrin was tested at concentrations of 1%, 2%, 5% in petrolatum. The frequency of skin irritation and sensitisation was low. Positive irritant reactions were only seen in 2 subjects (2 fenvalerate). Positive allergic reactions were only seen in 3 subjects (2

fenvalerate, 1 cypermethrin), and the one to cypermethrin was not considered clinically relevant. From the results in this study, one can conclude that pyrethroids only have a very slight irritant and sensitizing potential.

Epidemiological studies

A cross sectional survey on the prevalence of acute pyrethroid poisoning, including cypermethrin, in cotton workers was conducted by Chen et al. (1991). Effects of pyrethroid exposure were found in 26.8% of the spraymen (834 of 3113) and manifested as abnormal facial sensation, mainly burning and tingling which emerged as initial symptoms. Systemic symptoms included dizziness, headache, fatigue, nausea, loss of appetite, and apathy. None of these spraymen wore masks or gloves and most of them kept their upper extremities bare and wore only sandals during spraying. The area of dermal contamination of pyrethroids was, however, less than 30% of the body surface.

Expected effects of poisoning, aspects of diagnosis of poisoning

In humans, a variety of reversible symptoms have been reported (He et al., 1988, 1989).

The initial symptoms with occupational poisoning were burning, itching, or tingling sensation of the face, or dizziness that usually developed 4 to 6 hours after exposure. The skin symptoms could appear early after several minutes of spraying, followed by systemic symptoms as late as 48 h after exposure. After ingestion, the initial symptoms were mainly digestive such as epigastric pain, nausea, and vomiting, and developed within 10 minutes to 1 hour. Skin symptoms were not significant in patients with ingestive poisoning. The facial paresthesiae described following direct skin contact with cypermethrin, are highly characteristic for pyrethroids, and occur in the absence of any visible signs of skin irritation such as erythema, swelling, blistering, exudation, or desquamation.

The signs of systemic poisoning by cypermethrin, following massive ingestion, appear to be non-specific. Acute intoxications by pyrethroids have been reported to lead to signs and symptoms such as dizziness, headache, nausea, anorexia, fatigue, gastrointestinal complaints, and fever. In severe cases, exposure resulted in impaired consciousness, muscular fasciculations, convulsions, coma, and pulmonary oedema. A blood cholinesterase test might prove useful to exclude organophosphate poisoning.

First aid measures, therapeutic regimes

For acute systemic intoxication there is no specific antidote.

Most patients exposed require only skin or eye decontamination and symptomatic and supportive measures.

For paraesthesiae no specific treatment is generally required. However, topical application of vitamin E can reduce the severity of the skin reaction.

Convulsions should be treated with anticonvulsants such as diazepam (5-10mg if seizures are prolonged).

Induction of vomiting is not recommended following ingestion. Gastric lavage should also be avoided, since solvents present in many formulations may increase the risk of aspiration pneumonia. Alternatively, the administration of active charcoal 50-100g to an adult may be considered of a potentially toxic amount has been ingested within 1 hour.

3.12 SUMMARY OF HUMAN HEALTH EFFECTS ASSESSMENT, NOAEL AND AEL SETTING

3.12.1 Summary of human health effects assessment

ADME

Absorption of cypermethrin from the gastro-intestinal tract of the rat is rapid but incomplete. Urinary and faecal excretion was similar at the low dose (3 mg/Kg bw) for both the cyclopropyl and phenyl ring radiolabels, but at the higher dose (50 mg/Kg bw) faecal excretion predominated, especially in the males. This suggests that the absorption of cypermethrin is being saturated at the high dose. At the low dose 51.3 to 52.8% of the dose was absorbed by the male rats and 43.6% to 57.6% in case of the females. At the high dose level, 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. For the estimation of oral absorption, a conservative approach is adopted. Different values were adopted for animals and humans, based on the low dose (3 mg/Kg bw) data of the [redacted]. For **animals**, an oral absorption value of **44%** is adopted for deriving systemic NOAELs (PODs for the AELs are closer to the low dose rather than the high dose). For the estimation of **human** systemic exposure, an oral absorption value of **57%** is adopted.

Distribution. Following repeated daily oral dosing of 3 mg [^{14}C-phenyl]-cypermethrin, the levels of radioactivity in inguinal and peri-renal fat 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 (for both males and females), and the levels in the fat falling by 2-7 times: in males in peri-renal fat (2-fold), and in females in brown fat (7-fold).

Excretion. The excretion was rapid being virtually complete by 72 h following a single oral dose of [^{14}C-cyclopropyl]- or [^{14}C-phenyl]-cypermethrin at a dose of 3 or 50 mg/Kg bw. Urinary and faecal excretion was similar at the low dose for both radiolabels, but at the higher dose level faecal excretion predominated, especially in the males.

Metabolism. Hydrolytic cleavage of the ester bond and elimination of the *cis*- and *trans*-cyclopropanecarboxylic acid and 3-phenoxybenzyl moieties in the free and conjugated form is known to be a major route of metabolism in mammals, including humans. The cyclopropane carboxylic acid moiety is mainly and rapidly excreted as the glucuronide conjugate, with only limited hydroxylation of the methyl groups attached to the cyclopropane ring. The 3-phenoxybenzyl moiety is mainly converted to 3-phenoxybenzoic acid which is further metabolised to a hydroxyl derivative (3-(4'-hydroxyphenoxy)benzoic acid) and conjugated with glucuronic acid or sulphate. The major route of excretion of metabolites is via the urine. In faeces, most of the radioactivity is unchanged compound. The metabolism of cypermethrin is stereoselective with a preference for the *trans*-isomers (human and animal data).

Dermal absorption. The *in vivo* dermal absorption study in rats provided the most reliable dermal absorption data. The dermal absorption of cypermethrin determined in rats *in vi*vo resulted in an absorption of 7.6% and 12.7% of the applied dose for the concentrate (500 g/L) and spray dilution (25 mg/L). For the assessement of the human internal dermal exposure, a value of **13%** is used.

Absorption by inhalation. Pyrethroids are rapidly absorbed in humans following inhalation exposure, but no estimates are available regarding how much of an inhaled dose is absorbed for cypermethrin. Consequently, in the risk characterisation a value of **100%** absorption is used following inhalation exposure.

Acute toxicity

The oral toxicity of cypermethrin varies with the type of vehicle used and the isomer ratio. In general, aqueous suspensions were the least toxic and non-polar solutions the most toxic. The acute toxicity of the racemic mixture is also determined by the isomer ratio, with the *cis*-isomer found the most toxic (WHO, 1989). Oral LD_{50} values vary from 250 mg/Kg (in oil) to >5000 mg/Kg (in aqueous solutions). Inhalation LC_{50} = 3281 mg/m^3 (4h, aerosol, rat). Nevertheless, the toxic responses in all species were found to be qualitatively similar. The clinical signs observed after oral and inhalation exposure were indicative for an action on the central nervous system and consisted of salivation, ataxia, splayed gait, hyper-excitability to auditory stimuli, tremors, convulsions, choreoathetosis. These neurotoxic signs, better known as CS-syndrome, appear within 1 hour after dosing and survivors recover within 10-12 days. Transient facial sensory symptoms can appear after cypermethrin exposure. Abnormal facial sensations (burning sensations, tingling, tightness or numbness on the face) are reported in open literature, e.g. in health surveys (workers engaged in packaging cypermethrin), cross sectional surveys (field operators, spraymen). Cypermethrin was found of low dermal toxicity in the rat with clinical signs characterised by dyspnea, ruffled fur, curved and ventral body position. Dermal LD_{50} > 2000 mg/Kg bw (rat).

Our conclusions are based on the acute oral, dermal, and inhalation toxicity data obtained from studies performed with cypermethrin *cis:trans*/40:60. In conclusion, cypermethrin *cis:trans*/40:60 is of moderate acute oral and inhalation toxicity, but of low dermal toxicity.

Irritation

Cypermethrin *cis:trans*/40:60 is slightly irritant to the rabbit skin and eye, but does not require classification. Acute toxicity and repeated dose toxicity studies performed with rats revealed that cypermethrin has a respiratory irritation potential. Respiratory tract irritation caused by cypermethrin is characterised by cough, mild dyspnoea, sneezing, and rhinorrhea. This is confirmed with human data. Case reports reported shortness of breath, dyspnea, wheezing, cough, congestion, nasal discharge, burning eyes, after exposure (inhalation) of cypermethrin with the development of significant pulmonary dysfunction (still complaining of cough, congestion, wheezing) 7 months post-exposure.

Sensitisation

Cypermethrin *cis:trans*/40:60 was not found to be a skin sensitizer by animal testing (LLNA). However there are indications, from both animals and humans, that *technical cypermethrin* may have a mild skin sensitising potential. Results from preliminary experiments performed with technical cypermethrin (50:50) in rats indicated that technical cypermethrin had a weak skin sensitising potential. In addition, skin sensitisation (contact sensitivity and eczema) in humans is occasionally reported.

Respiratory sensitization is a recent endpoint introduced by GHS/CLP regulation. The toxicological effect of these active substance were discussed and approved for the inclusion of cypermethrin as an approuved active substance for PT8. No new data were provided for the PT18 assessment and the Human Health effects were not subjected to revision. Consequently, there is no data available to draw a conclusion for this endpoint.

Short/Medium-term toxicity

The medium-term *dermal* toxicity of cypermethrin was studied in a 21-day dermal toxicity study in rabbits. This resulted in irritation of the skin and was associated to systemic effects such as focal liver necrosis. NOAEL = 20 mg/Kg bw/d.

The medium-term *oral* toxicity of cypermethrin was studied in rats and dogs. The central nervous system and the liver were detected as the target tissue/organ. Neurotoxicity was characterised by clinical signs including piloerection, nervousness and uncoordinated movements, ataxia, splayed gait and hyperesthesia. In the dog, clinical signs of neurotoxicity were observed at 37.5 mg/Kg bw/d in a 90-day study (NOAEL = 12.5 mg/Kg bw/d). In the rat, clinical signs of neurotoxicity were observed at 80 mg/Kg bw/d in a 90-day study (NOAEL = 20 mg/Kg bw/d). In rats, neurotoxicity was confirmed by histopathology by peripheral nerve damage. (not in dogs). In addition, body weight was reduced, liver weight increased, and rats presented signs of anemia. In the open literature liver toxicity was characterised by inhibition of the rat liver ATPase activity. The oxidative stress induced by cypermethrin in the cerebral and hepatic tissues was evidenced by enhanced lipid peroxidation. Additionally, a decrease in delayed type hypersensitivity, leucopenia and immunotoxicity were observed when rats were dosed cypermethrin orally for 90 days at doses of 40 mg/Kg bw/d (NOAEL = 10 mg/Kg bw/d.

NOAEL medium-term = NOAEL (90-days, oral, dog) = 12.5 mg/Kg bw/d.

Long-term toxicity

The long-term *oral* toxicity of cypermethrin was studied in rats. The effects were in line with those observed in the medium-term studies. The central nervous system, liver, and kidneys were detected as the target tissues/organ. Hepatotoxicity was characterised by increased liver weight associated with microsomal enzyme activity induction, but not associated with histological lesions. Increased kidney weight was associated with an increase in blood urea.

NOAEL long-term = NOAEL (2-year, oral, rat) = 5 mg/Kg bw/d.

Carcinogenicity

Cypermethrin was tested in a combined chronic toxicity / carcinogenicity study in the rat. The overall results revealed no effect of cypermethrin treatment (0.05, 0.5, 5, 50 mg/Kg bw/d, orally) on the number and type of tumours.

Genotoxicity

Cypermethrin was found negative for genotoxic effects in *in vitro* bacterial and mammalian cell test systems (bacterial reverse gene mutation assay, mammalian gene mutation assay in L5178Y mouse lymphoma cells, mammalian chromosomal aberration study on CHO-cells). *In vivo*, cypermethrin did not produce micronuclei in the immature erythrocytes of the mouse bone marrow micronucleus assay (single oral dose), and was, therefore considered negative for mutagenicity.

Overall, the open literature provides inconsistent evidence of genotoxicity *in vitro* as well as *in vivo*. The data reported on the genotoxicity of cypermethrin are rather inconsistent, depending on the genetic system or the assay used. Most of these studies were not performed according to accepted guidelines. Additionally, they lack reliablility because of procedural flaws such as deviating route of administration, single versus repeated exposure, other sampling times, no use of positive controls, no 2nd or 3rd confirming experiments, no data about reaching the target organ. Nevertheless, the modest or marginal increases in DNA damage reported in some studies in peripheral lymphocytes or other cells indicate, at

least to a limited extent, potential genetic hazards posed by cypermethrin, and emphasize the need and the importance of protective measures and safety regulations to minimize exposure to cypermethrin.

Although the genotoxicity studies on cypermethrin cis:trans/40:60 did not exclude a potential for DNA damage, the global weight-of-evidence suggests that cypermethrin cis:trans/40:60 should not be considered a genotoxicant, and no classification as a Category 3 mutagen (under former DPD) or category 2 mutagen (under CLP) is warranted.

In addition, there was no evidence of carcinogenicity. Also in other repeated-toxicity studies, there was no evidence of proliferative lesions, which would possibly occur if cypermethrin cis:trans/40:60 would display aneuploidogenic or polyploidogenic properties in vivo.

Reproductive and developmental toxicity

The teratogenicity studies involving oral administration of cypermethrin during organogenesis at dosages up to 70 mg/Kg bw/d in rats and up to 120 mg/Kg bw/d in rabbits were without adverse effects upon the progress and outcome of gestation.

A three-generation study involving administration of the substance in the diet of the rat showed that cypermethrin exerts no effect on the different reproduction parameters or on the survival of the offspring. $NOAEL_{parental}$= 10 mg/Kg bw/d; $NOAEL_{reproductive}$= 50 mg/Kg bw/d; $NOAEL_{developmental}$= 10 mg/Kg bw/d.

According to the open literature, cypermethrin induced functional impairments at the neurotransmitter receptor levels in neonatal rats. However, since the multigeneration reproduction study in rats was without any indication of persistent effects in the offspring, which were also exposed to cypermethrin neonatally, it is suggested that receptor binding changes are not predicitive or causally related to the behavioural changes. Moreover, the most vulnerable phase for humans during the brain growth spurt is prenatal and not post-natal as in rodents. Therefore, exposure of the human foetus will be limited by maternal pharmacokinetics as well as maternal toxicity. The decreased male fertility seen in the rat and rabbit as demonstrated in the open literature appeared to be an indirect effect as it was caused at cypermethrin doses inducing clear general toxicity.

Based on the available data provided in the original dossier, there were no evidence giving rise to concern for an additional risk for the newborn or young humans that should trigger further investigations. According to results available to a similar substance, the WG-IV-2016 concluded that the applicant should provide a DNT study on cypermethrin six months before approval date of the active substance.

Neurotoxicity

Cypermethrin has a neurotoxic potential. Repeated oral dosing of adult laying hens with 1000 mg/Kg cypermethrin produced no immediate or delayed signs of poisoning, nor any histopathological lesions in the nervous system. However, the hen sciatic nerve is not be suitable for studying pyrethroid-induced nerve damage. In contrast with hens, rats treated with a single dose of cypermethrin (60 mg/Kg bw) showed behavioral changes indicating a broad neurological activity of cypermethrin. A NOAEL was observed at 20 mg/Kg bw. The clinical signs observed are characteristic for the acute poisoning with a type II pyrethroid: choreoathetosis accompanied by salivation (CS syndrome).

In the rat, cypermethrin also produces epileptic activity during repeated administration. The neurotoxic effect of cypermethrin on peripheral nerves (axons, endoneurium) was highly correlated with exposure

time. Cypermethrin exerts its toxicity by opening the voltage-gated sodium channel slowly for extended times, leading to a prolonged sodium current in the target neurons. Furthermore, the decrease in the Na^+, K^+-ATPase pump activity is involved in the paroxysmal epileptic activity induced by cypermethrin. Cypermethrin also inhibits $GABA_A$ receptors.

The post-approval data for Cypermethrin PT18 was provided by the applicant, Arysta Life Science Benelux sprl, via R4BP3 on 29.11.2019 (case: BCKM055469-19), containing 2 neurotoxicity studies:

- [redacted] *Cypermethrin: Oral (Gavage) Screening Study of Reproduction/Development Toxicity in the Rat. (OECD 421).*
- [redacted]. *Cypermethrin: Oral (Gavage) Study of Developmental Neurotoxicity in the Rat (OECD 426).*

In both studies, clinical effects were observed on parents at dose 5 mg/kg bw/day and more. Salivation, mouth rubbing and paddling of the forelimbs are reported, mainly immediately after administration (by oral gavage). It was concluded at the WG III 2023 that these effects have to be considered as adverse and that the parental LOAEL is set at 5 mg/kg bw/day.

Regarding the offspring, effects on learning and memory and in the functional observational battery were observed at dose 25 mg/kg bw /day in the OECD 426 study. Since only the top dose was tested, it was agreed to not set a NOAEL, but only a developmental LOAEL of 25 mg/kg bw/day.

Other: Immunotoxicity

Cypermethrin causes immunosuppression: both the humoral and cell-mediated immune response are impaired by cypermethrin.

Other: Endocrine disruption activity

The estrogenic potential of cypermethrin cis:trans/40:60 based on ER-mediated mechanisms remains equivocal. Contradictory results were revealed in different studies. In summary, the estrogenic and antiandrogenic effect of cypermethrin cis:trans/40:60 (and pyrethroids in general) depend on the assays or cells used. Results indicate that data obtained with high concentrations (> 10 µM) should be interpreted carefully (solubility of test chemical, cell toxicity). Possibly, cypermethrin cis:trans/40:60 is an estrogen-like chemical that might act through signalling pathways other than direct ER binding, and as such, might function as an endocrine modulator. However, at present no definite conclusions can be drawn.

In November 2016 , the criteria for identification of endocrine disrupters are still under discussion for the biocide regulation. The entry into force is foreseen for 2017.

Therefore, eCA suggest to to consider the available studies at the renewal stage of cypermethrin for PT8 or PT18.

3.12.2 NOAEL setting

Table 3.11: Summary of relevant NOAELs for risk characterisation

Study	Critical effect	LO(A)EL and NO(A)EL
Rat, acute oral		Cis:trans/40:60 LD50 = 1732 mg/Kg bw (arachis oil) LD50 = 500 mg/Kg bw (groundnut oil) Cis:trans/50:50 LD50 = 287 mg/Kg bw (10% in corn oil) Cis:trans/37:63 LD50 = 250 mg/Kg bw (corn oil)
Rat, acute dermal		LD50 > 2000 mg/Kg bw (40:60)
Rat, acute inhalation		LC50 = 3281 mg/m³ (40:60)
Rat, acute delayed neurotoxicity study	Behavioral effects	LOAEL = 60 mg/Kg bw/d **NOAEL = 20 mg/Kg bw/d (corn oil)**
Rat, oral, 90-day study	Neurotoxicity: evidenced by clinical observations, confirmed by histopathology by peripheral nerve damage. Liver and kidney toxicity Decreased bw gain	LOAEL = 80 mg/Kg bw/d NOAEL = 20 mg/Kg bw/d
Dog, oral, 90-day study	Neurotoxicity: evidenced by clinical observations, but *not* confirmed by histopathology. Liver toxicity Decreased bw gain	LOAEL = 37.5 mg/Kg bw/d **NOAEL = 12.5 mg/Kg bw/d**
Rat, oral, 2-year study	Neurotoxicity Liver and kidney toxicity Decreased bw and food consumption	LOAEL = 50 mg/Kg bw/d **NOAEL = 5 mg/Kg bw/d**
Rat, 3-generation study	Paternal: Decreased bw gain and food intake. No evidence of any neurotoxic responses. Offspring: Reduced litter size and pup weight at parental toxic doses. Fertility: not affected.	$LOAEL_{parental}$ = 50 mg/Kg bw/d $NOAEL_{parental}$ = 10 mg/Kg bw/d $LOAEL_{developmental}$ = 50 mg/Kg bw/d $NOAEL_{developmental}$ = 10 mg/Kg bw/d $NOAEL_{reproductive}$ = 50 mg/Kg bw/d
Rat, developmental study	Maternal: Decreased bw gain at 35 mg/Kg bw, transient neurological disturbances at 70 mg/Kg bw/d. Developmental: No effects at maternal toxic doses	$LOAEL_{maternal}$ = 35 mg/Kg bw/d $NOAEL_{maternal}$ = 17.5 mg/Kg bw/d $NOAEL_{developmental}$ > 70 mg/Kg bw/d
Rabbit, developmental study	No effects (maternal, developmental)	$NOAEL_{maternal}$ > 120 mg/Kg bw/d $NOAEL_{developmental}$ > 120 mg/Kg bw/d
Rat, screening study of reproduction/development toxicity	Maternal: Salivation, mouth rubbing and paddling of the forelimbs at 5 mg/kg bw/day Developmental: 3 total litter loss at 25	LOAEL parental: 5 mg/kg bw/day LOAEL developmental: 25 mg/kg bw/day NOAEL developmental= 5 mg/kg bw/day

	mg/kg bw/day	
Rat, Developmental neurotoxicity study	Maternal: Salivation, mouth rubbing and paddling of the forelimbs at 5 mg/kg bw/day Developmental: Effects on learning and memory and on FOB at 25 mg/kg bw/day	LOAEL parental: 5 mg/kg bw/day LOAEL developmental: 25 mg/kg bw/day

3.12.3 AEL setting for cypermethrin 40:60

Systemic AELs derived for acute, medium-term, and long-term exposure starting from points of departure (PODs).

Assessment factors: WG III 2023 agreed on assessment factor of 200 (100 for inter & intraspecies and additional 2 for using LOAEL value instead of NOAEL).

The long-term AEL was not derived with the NOAEL long term but was aligned with the acute and medium term AEL which were lower. The HH WG III 2023 considered the PPPR assessment too conservative, due to the application of stricter AF, and derived the reference values differently. It was decided to abstain from incorporating the values set under PPPR, in light of the one substance one assessment approach.

Acute AEL

POD: LOAEL oral, DNT study, rat, 5 mg/kg bw/day

Oral absorption: 44%

AF: 200

Acute AEL $_{systemic}$ = 0.011 mg/Kg bw/d

Medium-term AEL

POD: LOAEL oral, DNT study, rat, 5 mg/kg bw/day

Oral absorption: 44%

AF: 200

Medium-term AEL $_{systemic}$ = 0.011 mg/Kg bw/d

Long-term AEL

POD: LOAEL oral, DNT study, rat, 5 mg/kg bw/dayOral absorption: 44%

AF: 200

Long-term AEL $_{systemic}$ = 0.011 mg/Kg bw/d

3.12.1 ADI and ARfD setting for cypermethrin 40:60

The following values for ADI (acceptable daily intake) and ARfD (acute reference dose) were agreed at WG-III-2023

ADI = 0.025 mg/kg bw/day

ARfD = 0.025 mg/kg bw/day

4 ENVIRONMENTAL EFFECTS ASSESSMENT

In this Section, summaries and evaluation of data presented in Doc.III-A7, Eco-toxicological studies of the CA-Report are reported as far as possible in summary tables.

The data highlighted by the use of a grey bacKground in the tables are from studies where a full (robust) **STUDY SUMMARY** made in accordance with the Technical Notes for Guidance on Dossier Preparation was available, i.e. the **KEY STUDIES.** Summaries of the rest of the studies are available as **IUCLID** entries only.

The data provided fulfil the core and additional data requirements for both the active substance and the representative biocidal product for the field of use applied for.

Unless otherwise stated, all studies were made according to internationally accepted guidelines and principles for Good Laboratory Practice (GLP).

The allocation of reliability scores 1 or 2 to the Key Studies indicates that results from such studies can be considered for risk assessment. Supplementary studies (i.e. studies with a reliability score of 3 or more, or even 0) give additional information for the risk assessment and are available as IUCLID entries.

For every Eco-toxicological end-point a proposal for classification/Labelling according to the criteria in Directive 67/548/EEC is given.

Cypermethrin possesses three chiral carbon atoms and is therefore a racemic mixture of 8 isomers (four *cis*- and 4 *trans*-isomers). The technical products commonly available contain more than 92% cypermethrin and the ratio *cis*- to *trans*-isomers varies from 50/50 to 40/60.

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* (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) However, [redacted] did not found this relation for the brain toxicity of cypermethrin to fish.

Weipung L. *et al* (2004) showed that isomer selectivity in degradation by bacteria isolates and sediments also occurs. The -cis enantiomers being degraded at slower rate in comparison to the -trans enantiomers. Of the two biologically active enantiomers, 1R-cis α S was relatively persistent compared with the other stereoisomers, whereas 1R *trans* α S was likely the least persistent among all stereoisomers. Therefore, the difference between 1R *cis* α S and 1R *trans* α S in persistence may be compensatory and the overall persistence of the biologically active enantiomers may be similar to the overall trends of all Cypermethrin stereoisomers.

Thus in the case of cypermethrin, the active components are 1R cis α S and 1R trans α S, e.g. approximately 25% of the mixture. Less active isomers are 1R cis α R; 1S cis α S ; 1R trans α R and 1S trans α S e.g. approximately 50% of the mixture . Relatively non-active isomers are 1S cis α R and 1 S trans α R e.g. approximately 25% of the mixture.

In the following, tests were performed with cypermethrin (40:60) as stated in chapter 1. Studies performed after 2005 are coverd by certificates of analysis and test item fulfil the purity requirements. For older studies, a certificate of analysis is not always provided.

4.1 FATE AND DISTRIBUTION IN THE ENVIRONMENT

4.1.1 Degradation

4.1.1.1 Abiotic degradation

4.1.1.1.1 Hydrolysis

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 results are expressed in the table 4.1.1.1.1

Table 4.1.1.1.1

Guideline/ Test method	pH	Temperature (ºC)	Initial concentration (µg/L)	Reaction rate constant, Kh	Half-life, DT50 (days)	References
EEC method C.7	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 According to the results of this screening test (23-26°C)	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.						

The study shows that cypermethrin hydrolysis is pH dependant. Cypermethrin 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 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 page 49 of the TGD the respectives half-life corected for 12°C becomes 98.8 d for pH 7 and 1.65 day at pH 9. According to these last values, the respectives pseudo first-order rate constant can be calculated to be KpH 7 = 0.007 d-1and KpH 9= 0.42d^{-1}

Conclusion:

Conclusion for hydrolysis: Cypermethrin *cis:trans*/40:60will 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).

4.1.1.1.2 Photolysis in water

Photolysis in water was addressed in two independent studies on cypermethrin by Greenwood, J.and Maudsley, L. (2003) and by Swales, S. (2003)

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.

Table 4.1.1.1.2(1)

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	0.004µg/mL	68% after 90 hours	0.0308	Greenwood, 2003, Covance report no 0040/034

Analysis by Thin Layer Chromatography (TLC), illustrated that after 90 hours irradiation, the majority of radioactivity detected was cypermethrin, 68% of applied radioactivity at 90 hours. The levels of origin material decreased 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.

In the second study by Swales(2003) the photodegradation rate of [14C] cypermethrin (cis:trans 40:60) was studied by 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).

Table 4.1.1.1.2(2)

Guideline / Test method	Isomer	Initial concentration	Total recovery of test substance (% of applied)	Photolysis rate constant (Kcp)	Direct photolysis rate constant (per day)	Stability	Half-life (t½E) (days)	Half-life (d), dark control	Reference
EC directive 94/37/EC Section 2.9.2	(14C Phenoxy) cypermethrin	0.004µg/mL	96-105%.	0.078	0.0469	-	14.7	22.1	Swales, 2003, Covance, report no. 40/35-D2149 CYP/M70.Greenwood, 2003, Covance report no 0040/034
	(14C cyclopropane) cypermethrin	0.004µg/mL	96-107%	0.0976	0.0557	-	12.4	16.5	
Main photolytic degradants: 3-Phenoxybenzoic acid (15%) and DCVC acid (18%) of applied radioactivity.									

Degradation process in both the irradiated solution and controls was first order. The half-life in the irradiated solution was 8.85 summer sunlight days for the (14C phenoxy) cypermethrin and 7.10 summer sunlight days for the (14C 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 d for ^{14}C phenoxy label and 0.0557 d^{-1} and 12.4 d for ^{14}C cyclopropane label. The main photolytic degradants was 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.

4.1.1.1.3 Photolysis in air

Photolysis in air was investigated by the mean of the EPIWIN AOP model. EPIWIN AOP v1.91 gives an indirect photolysis rate constant of 0.02326*$\mathbf{10^{-17}}$ cm3/molecule-sec; half-life = 0.749 days (24-hr day; 0.5E6 OH/cm3) or 17.990 hours (also referenced in Greenwood, 2003, Covance report no 0040/034).

Atmospheric risk: Cypermethrin has a low volatility and emissions to the air compartment are expected to be low.

Global warming: Data not available on absorbance in so-called atmospheric window (800-1200nm). However, the vapour-pressure is so low, together with an atmospheric half-life less than one year that this is not considered to be an issue.

Stratospheric ozone: AOP v1.91 gives an overall ozone rate constant = 0.02326 * 10^{-17}cm3/molecule-sec; half-life 49.27 days and according to the TGD on risk assessment (Part II, Section 3.7.2) ozone depletion values approach zero for molecules with atmospheric half-lives less than one year.

Tropospheric ozone: According to the TGD on risk assessment (Part II, section 3.7.2) there is at present no procedure available to estimate the effect on tropospheric ozone if only the basic characteristics of a substance are known

Acidification: It is possible that oxidation of cypermethrin will lead to formation of Nitrogen-containing oxides. However, due to the low expected emissions to the air compartment, it is not expected that this will have an effect on acidification of the receiving soil or surface water.

Conclusion for photodegradation in air: Cypermethrin has an estimated half-life of 17.990 hours (indirect photolysis, OH) and 49 days (indirect, ozone). It is predicted not to have potential as a greenhouse gas.

4.1.1.1.4 Photolysis on soil

The photodegradation of (14C phenoxy) cypermethrin and (14C 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. [14C phenoxy] cypermethrin (40/60) and [14C 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.

Table 4.1.1.1.4

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	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 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, 2003,Covance report number 40/44,

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 (6% AR) and DCVC acid ((2,2-dichlorovinyl)- 2,2- dimethylcyclopropanecarboxylic acid) (3% 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 DT90 values were 201 and 230 days (light samples) for the (14C phenoxy) cypermethrin and the (14C 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 (14C phenoxy) cypermethrin and 2.5 days for the (14C 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.

4.1.1.2 Biodegradation

4.1.1.2.1 Ready biodegradability

Table 4.1.1.2.1

Guideline/ Test method	Test type	Inoculum		Test substance concentration.	Degradation		Reference
		Type	Adaptation		Incubation period	Degree (%)	
OECD 301B	Ready Biodegradability (modified Sturm test)	Activated sludge, domestic	No	10mg/L and 20mg/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 from OECD 302C.	Inherent biodegradability.	Activated sludge	No	30mg/L	28 days	0	Burwood, 2005,Covance Report no 1669/017-D2149
BS method 6068 and ISO method 11734 (1995)	Anaerobic (ultimate) biodegradability	Mineral salts medium (MSM) and pre-digested anaerobic sludge inoculum, domestic	No	20mg as [C]/L	60 days	17%*	Barnes, 2005, Huntingdon Life Sciences, HZL 010/053287

* Indicative value

Cypermethrin was tested for the ready biodegradability by Klein, in 1990. Initial cypermethrin ((RS)-a-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 CO2-free air. The extent of biodegradation was determined by titration of the total CO2 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 CO2 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.

4.1.1.2.2 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, CO2 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 CO2-free air. The exhaust air was passed through a series of CO2 traps containing a barium hydroxide (Ba(OH)2) solution. The entire content of all traps was titrated with acid to determine the quantity of CO2 produced.

By the end of the test, Cypermethrin *cis:trans*/40:60 did not show any evidence of biodegradation during the test. It was noted that CO2 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**.

4.1.1.2.3 Ultimate biodegradability

In a third test to assess the biodegradation of cypermethrin, 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 degested sludge-Method by measurement of the biogas porduction. 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. 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 inverted 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.

4.1.1.2.4 Water/sediment degradation

Brice, (2005) has 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 dropwise 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 analysed. 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.

Table 4.1.1.2.4

Guideline/ Test method	Test type	Inoculum		Test substance concentration.	Degradation		Reference
		Type	Adaptation		Incubation period	Degree (%)	
OECD 308	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 DT50 comprised between 10.9-14.3 days 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	Brice 2005,Covance report 1669/014,

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 C0 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 [14C-phenoxy] cypermethrin and [14C-cyclopropyl] cypermethrin were ≥90% at each sampling interval, with one exception of 89% at 100days 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 2.9d at 20°c for the trans isomers and 12.5 to 16.9 d for the cis-isomer which correspond to 2.1 to 5.5d and 23.7 to 32d 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 dediment 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 timepoint. 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 4.1.1.2.4 (02) Bound residue.

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.

4.1.1.2.5 Aerobic degradation in soil

The route and rate of degradation of cypermethrin was studied in one soil type (soil PT102) at 20 ± 2°C by Brice A. and Cooke C. (2005). The rate of degradation of cypermethrin *cis:trans*/40:60 was also studied in this soil at 10 ± 2ºC, and in three other soil types at 20 ± 2ºC. 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 ± 2ºC 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 dropwise 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 ± 2ºC.

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.

Table 4.1.1.2.5 degradation of cypermethrin in soil

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	¤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, 2005, 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)	
	¤SK920 191 (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)	
	¤SK155 56090 (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

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% at the terminal timepoint. Volatile radioactivity (carbon dioxide) increased to 49 to 78% of applied radioactivity at the terminal timepoint. $^{14}CO_2$ 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 4.1.1.2.6: Degradation rates for cypermethrin metabolites at 20°C

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 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 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 caractérised by DT_{50} of 9.7- 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 rescpectively. 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 cypermthrine. The

arithmetical mean DT $_{50}$ at 20°C is 13.45d which correspond to 25d à 12°C. This latest value was used in the refinemment of the assessment in doc II B for PT 8. No refinement was made for PT 18.

Table 4.1.1.2.7 Degradation rate of total cypermethrin at 20°C

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 4.1.1.2.8 Degradation rates for cis and trans-cypermethrin at 20°C

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 4.1.1.2.8

In conclusion, **cypermethrin** cis:trans*/40:60* was metabolised 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 mineralisation to carbon dioxide.

4.1.1.2.6 Anaerobic degradation in soil

The route and rate of cypermethrin *cis:trans*/40:60 was studies 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. 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 lebvels of cypermethrin and its metabolites. For the determination of DT50, DT75 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). DT50; DT75; and DT 90 were calculated from the equations of the lines.

Table 4.1.1.2.6.1 Dedradation rate of cypermethrin and cypermthrin isomers

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)							Brice, A., Cooke, C. (2006); Covance Laboratories Limited, report no. 1669/013-D2149, 21
	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 4.1.1.2.6.2 recovery rate of applied radioactivity on cypermethrin and cypermethrin isomer respective to metabolites occurence

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	Surface water	Ph*	ND/ND	ND	ND	NA	16.6 (120)	ND	NA	NA	Brice, 2006, 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 (120)	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)	

ND= not detected; NA, Non-applicable

Cypermethrin was metabolised 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 initally applied radioactivity fort the phenoxy and the cyclopropyl cycle respectively and mineralisation to carbon dioxide.

Table 4.1.1.2.6.3 Reflux extract and bound residues.

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 is indicated in the above table

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.

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In conclusion, Cypermethrin 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.

4.1.2 Distribution

4.1.2.1 Adsorption / desorption in soils (screening test)

The adsorption and desorption properties of Cypermethrin *cis:trans*/40:60 was evaluated y Brice (2005). The adsorption characteristics of [14C]-cypermethrin were determined in four soil types and one sediment according to OECD Guideline 106 (January 2000).

Table 4.1.2.1 Summary

Guideline/ Test method	Temperature (ºC)	Radioactive labelling	Test substance conc. (µg/L)	Soil	Material balance	Reference
OECD 106	20±2°C	Yes. [14 C -phenoxy]-cypermethrin cis and trans isomers.	2.0µg/ml	5 types see table 4.1.1.3.2	Soil = 0.6-1.3% Supernatant = 96.8-101.1%.	Brice 2005, covance study 1669/015

Table 4.1.2.2 Soil and sediment characteristics

Soil type	Sand (%)	Silt (%)	Clay (%)	Organic C (%)	pH	Reference
EL-7 (clay loam)	31	45	24	5.2	6.3	Brice 2005, 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 [14C]-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, 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 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 table 4.1.2.3).

Table 4.1.2.3 Estimated Koc

Soil type	Koc	*Kd	Reference
EL-7 (clay loam)	≥202418	-4858	Brice 2005, 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)	2,676,776 - 4,586,002	/	

According to the Qsar in the TGD (first Qsar of the table 4 TGD partIII, page 26) the value for Koc is 2,676,776 and 4,586,002 for Log Pow 5.3 to 5.6, which confirms that the result of the test only provide low values for Koc, and support the used of the highest Koc value within the minimums availlable to derive Ksup, water

Conclusion: These results indicate that the minimum Koc values ranges from 80653 to 574360 in the four soils and is minimum 527972 in the sediment. In the risk assessment the minimum Koc value for the soil SK566696 (loamy sand) rounded to 575 x10E03 has been used to derived $K_{susp, water}$

Koc= 575 x10E03

4.1.3 Conclusion of fate and distribution:

Cypermethrin *cis:trans*/40:60 is:

Stable in water in acidic condition (>1year at 50°C) relatively stable at neutral pH (7) but hydrolysed at alkaline pH (9) at 12°C

Is photolysed in water, in the air and in soil

Not ready biodegradable

Not inherently biodegradable

Not ultimately biodegradable

Degradable in a water-sediment compartment

Métabolised in soil under aerobic condition in three metabolites

Métabolised under anaerobic condition in three metabolites

Is characterised by a minimum koc of 575 x10E03

4.2 EFFECTS ON ENVIRONMENTAL ORGANISMS

4.2.1 Aquatic compartment

4.2.1.1 Acute toxicity to fish

Guideline/ Test method	Spp.	Endpoint	Exposure		Results (µg /L) measured			Reference
			design	duration	LC_{50}	LC_{100}	NOEC	
OECD 203	Onchorhynchus mykiss	Death	Semi-static	96 hour	2.83	4.11	n.d.	[redacted]

[redacted] 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.

The test item concentrations where monitored for freshly prepared media at 0 and 72h and for old test media at 24 and 96h. As some of the value where outside the 80-120% of the nominal range, 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 4.11 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 µg/L.

Conclusion:

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

4.2.1.2 Acute toxicity to invertebrates

Guideline/ Test method	Species	Exposure		Results (µg a.s./L) measured			Reference
		design	duration	EC50	EC100	NOEC	
OECD 202	Daphnia Magna	Static	48-hr	4.7	Could not be determined.		Manson, 2005, Covance report 1669/019-D2149

In a second study, Manson 2005 has evaluated the effects of cypermethrin *cis:trans*/40:60 on *Daphnia magna* according the 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 100µg/L.

The test item concentrations were measured at the beinning 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 of 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, with a corresponding no observed effect concentration (NOEC) of 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).

4.2.1.3 Growth inhibition on algae

Guideline/ Test method	Spp.	Exposure duration	Results (µg a.s./L) measured			Reference
			EbC50	ErC50	NOEbC	
OECD 201	Pseudokirchneriella subcapitata (formerly Selenastrum capricornutum)	96 hours	>33.0µg/L	>33.0µg/L	= or >33.0µg/L	Manson, 2006, Covance report 1669/020

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 ErC50 and the EbC50 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 ErC50 and EbC50 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.

4.2.1.4 Inhibition of microbial activity (aquatic)

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*/40:60in emulsifier surfactant was used.

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

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* show 50 % inhibition of the microbial repiration at effective concentration of 163 mg/L

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

4.2.1.5 Chronic toxicity to fish

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

[redacted] and [redacted], 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)* over embryo development, hatching and for 28 days post-hatch, in accordance with OECD (July 1992) Guideline 210 and OPPTS 850.1400 Fish Early-Life Stage Test.

Both tests were conducted under continuous flow conditions, with embryos (respectively less than 25 h old and 24h on exposure to the test solutions). In the first study (2005) larvae/fry were exposed to the following nominal concentrations of [14C]-Cypermethrin; 0.01, 0.032, 0.1, 0.32 and 1 µg/L. In the second study, larvae and fry were exposed to non radiolabeled Cypermethrin at the following nominal concentration; 0.005, 0.015, 0.045, 0.135, 0.405 and 1.22µg/L. Both solvent and non-solvent controls were included in the tests. Duplicate tanks were tested at each concentration.

Guideline/ Test method	Spp.	Endpoint	Exposure		Results (µg a.s./L) measured			Reference
			design	duration	Effect	NOEC	LOEC	
OECD 210	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	LOEC (fry survival) 0.9	[redacted]
OECD 210	Pimephales promelas	Fry survival, Body length, Body weight	Flow through	Embryo-development, hatching and 28 days post hatch	Hatching success ranged from 70to 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]

[redacted]

On each day of the study, commencing 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 success - 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 as a result of a contamination (biological effect) rather to a chimical effect of the molecule. An empirically derived NOEC would be regarded as 0.11 µg/L nominal (0.089 µg/L based on mean measured concentrations).

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 effects - 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.

[redacted]

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 Success -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 97 - 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 suffers of deficiencies which rend difficult the analysis of the results. The TM II 2011 concluded that the study is not sufficiently robust and therefore a conservative approach should be adopted. Based on the above parameters, an overall NOEC of 0.01 µg/L measured has been established.

The second study adresse the point more accurately, suffers no deficiencies and provide a robust NOEC of 0.463 µg/l.

Therefore eCA suggested to disregards the study of [redacted] and to derive the NOEC from the study of [redacted]. This proposal was agreed during BPC- WG IV 2016. The proposed value should be added in the LOEP and the summary added in the doc III A.

NOEC Fish = 0.463 µg/L

4.2.1.6 Chronic toxicity to aquatic invertebrates

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

Guideline/ Test method	Spp.	Exposure		Results (µg a.s./L) measured			Reference
		Design	duration	LOEC	NOEC	EC50 (21-d, reprod)	
OECD 202, part II. Reduction in reproduction test.	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)

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, 0.040, 0.200 and 1.000µg/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 (0.8mg/L). Results are express as reduction in reproduction. The NOEC and EC_{50} were calculated based on the nominal concentration.

Conclusion:

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

4.2.2 Bioaccumulation

[redacted] 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.

Guideline/ Test method	**Species.**	**Test material conc. (mg/L)**	**Radio-labelled**	**Exposure**		**BCF**	**Elimination**	**Remarks**	**Reference**
				design	**duration**				
OECD guideline (1981) part 305E	Rainbow trout	0.1ppb	No	Flow-through	10 days	373± 45	Depuration rate constant = 0.00158 - 1/hr	Not bioaccumulative	[redacted]

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 rapidely until the 160th hour and further but small increase could be observed till the end of the uptake phase. In

spite of log Pow of 4.47 which would predict a steady state reachable within 85hs, it tooks 240hs 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 Kow = 5.6) according to the equation (74 page 126 of the TGD) may be explained by the duration of the uptake phase wich were only 10 days and by the addition of the solibiliser.

For reference, in the DAR from the PPP regulation a study on bioaccumulation provided a BCF of 1204 L/Kg wet wt.

Both studies can not be used as key study due to lack of realiability for the first one and due to no access of the applicant for the second study.

The QSAR BCFwin from the tool suit EPISUITE will be used 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.

Using BCFwin (EPISUITE) for a Log POW of 5.45, a LOG bcf of 2.62 is calculated which correspond to a BCF of 417L/Kg wet-wt (Data-Base Structure Match: Cypermethrin) The LOG BCF calculated based on the Log Pow with correction for cyclopropyl esther (-1.259) is 1.733 which correspond to a BCF of 54.11L/Kg ww.

Conclusion:

Cypermethrin *cis:trans*/40:60 tends to bioaccumulate in organism with a typical bioaccumulation factor of 417 L/Kg.

4.2.3 Toxicity to other non-target aquatic organisms: mesocosm study

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 EC50 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 where concentrations were 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 :

Summary of NOEC values < 1.0 µg a.s./L, including an overall NOEC/NOAEC, for Zooplankton

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	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 inany 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 (PCR) 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 PCR 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 :

Summary of all NOEC values < 1.0 µg a.s./L (NOEC = 1 µg/L in the empty boxes) for Emergent insects

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 NOECcommunity 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 :

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).

Summary table of all NOEC values < 1.0 µg a.s./L (NOEC = 1 µg/L in the empty boxes) for Macrozoobenthos

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

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 of the 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.

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

Summary table of all NOEC values < 1.0 µg a.s./L for Periphyton

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 caracterised by an NOAEC of 1.0 µg/L.

4.2.4 Toxicity of metabolites

The major degradation pathway of cypermethrin in water, soil, plants, insects, birds and fish consists in the cleavage of cypermethrin into a cyclopropane carbonic acid and dibenzyl (3-phenoxybenzoic acid) moiety (= 3pba). In these degradation studies, DCVC acid accounted for up to 40% of the applied dose in water, 17.4% in soil and 33.4% in plants (as conjugate in this latter case).

Maximum percentage of cypermethrin degradation product identified in degradation studies

	3-PBA	DCVC	3 PBAD
Photlyse in water*	15%	18%	3%
Photolyse in soil*	6%	3%	/
Water	21%	38%	/
Sediment	29%	20%	/
Aerobic soil degradation	10.2%	17.5%[1]	
Anaerobic degradation soil	35.1%	31.2%	0.7

*irradiated samples
[1]Trans-DCVC + Cis-DCVC

The relevant metabolites are 3-PBA and DCVC. Whith such high concentrations of metabolites rapidly found in the various metabolism/ degradation studies, one can consider that its toxicity is covered by the studies on cypermethrin (Evaluation report on the equivalence; Agriphar Confidential 2007).

4.2.5 Toxicity to terrestrial organisms, plants

A GLP test according to the OECD 227 guideline was performed to test the toxicity of cypermethrin *cis:trans*/40:60 to plants.

This vegetative vigour test study was designed to evaluate the acute toxicity of Cypermethrin 10g/L ME to non target higher plants at a single treatment rate to determine if further testing (dose response test) is needed.

Twelve 2-leaf stage plants of 6 different species (*Beta vulgaris, Cucumis sativus, Glycine max, Helianthus annuus, Avena sativa and Alium cepa*; two monocotyledonous and four dicotyledonous) were treated at 15 l product/ha nominal application rate of the test item. The results were compared to application of demineralised water control. Each treatment group consisted of 6 (monocotyledonous) to 15 (dicotyledonous) replicates containing respectively two and five plants. In total, 30 plants per groups and species were treated. The test duration was 21d after application. The test temperature range between 21-27°C; the relative humidity ranged between 55-95%; the photoperiod was 16/8h/d and the light intensity varies from 5 to 16. The plant dry weight was determined after test termination. Statistics were done on the plant dry weight.

No phytotoxic effect were observed for all species but *Heliantus annuu*, where slight effect (<20%) chlorosis were observed.

Conclusion

The study provided has a low reliability for a biocide purpose due to the single application of a single dose of the test item which is a diluted product. However, the result clearly indicate that at the application rate of cypermthrin 10 g/L ME on non target plant species few toxicity effect occurs unless slight chlorosis in one species. No dose response curve has been established in this study and it is also difficult to estimate which dosis each single plant has received. Therefore it is impossible to set even a NOEC from the result of this study.

In the absence of any phytotoxic effects resulting from the use of cypermethrin cis:trans/40:60 in agriculture for decades, the weight of evidence of the historical use of cypermethrin cis:trans/40:60 in agriculture is a reasonable argument for the statement of no phytotoxicity of cypermethrin to plant.

4.2.6 Toxicity of cypermethrin to terrestrial organisms, earthworms

4.2.6.1 Acute toxicity to earthworms

Inglesfield, C.(1984) and Dickhaus, S.(1989) has both evaluated the effect of cypermethrin *cis:trans*/40:60 on earthworms. The study by Inglesfield was contucted with a protocol close to the OECD 207 (this protocol was part of the proposal for the settings of OECD 207). The result shows that the LC_{50} is bigger than 100mg/$Kg_{soil.}$ In this study, only mortality was recorded and no monitoring of the test substance was performed at day 14. A twa mean concentration could be calculated but considering the short duration period of the test and the result based on nominal concentration (>100mg/Kg) the outcome of the recalculation would certainly not change the result of the risk assessment.

The second test performed by Dickhaus, S. (1989) according to OECD Guideline for Toxicology of Chemicals (1981); BBA-Guideline (1982) and appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, FDA (1959).

No monitoring of the test item was made and the results are based on the nominal concentration of the test substance applied to the soil at study initiation. The results confirm the findings of the first study with a LC_{50} = 575mg/Kg_{soil}.

Guideline/ Test method	Spp.	Endpoint /type of test	Exposure		Results (mg a.s./Kg) measured		Reference
			design	duration	LC50/EC50	NOEC	
OECD 207	Eisenia foetida	mortality	/	14 days	EC50>100mg/Kg substrate	100 mg/Kg substrate	Inglesfield, 1984, Bull. Environ. Contam. Toxicol.) 33: 568-570
OECD guideline (1981)	Eisenia foetida	mortality	Test substance incorporated (mixed) in the substrate	14 days	EC50 575mg/Kg substrate	100 mg/Kg substrate	Dickhaus, S. (1989) Pharmatox Beratung und Forschung GmbH, Germany; report no. EH/B.1-7-96-89 (CYP/T127)

4.2.6.2 Chronic toxicity to earthworms

Servajean E (2011) has studied the effects of cypermethrin on the earthworm's reproduction with Cypermethrin according to the OECD 222 test guideline. The test was performed according to GLP and the final test report is well documented.

Guideline/ Test method	Spp.	Endpoint /type of test	Exposure		Results (mg a.s./Kg dry soil) measured		Reference
			design	duration	EC50 reproduction	NOEC Mortality biomass reproduction	
OECD 222	Eisenia foetida	Mortality Biomass reproduction	Test substance incorporated (mixed) in the substrate	8weeks	EC50 :22.5	100 (mortality) 30.8(biomass) 5.2 (reproduction) 4.0 (geom reprod)	Servajean, E. (2011); Earthworm reproduction test with Cypermethrin; Phytosafe s.a.r.l., France, report no. 11-99-064-ES, 30th November 2011

In this study, the effects of cypermethrin cis/trans: 40/60 was evaluated on mortality, biomass changes and number of juvenilles. Ec_{50} reproduction was evaluated. A range-finding test was first performed as an acute toxicity test, using one replicate unit of 10 worms for each of five test item treatmentsof 0.1, 1.0, 10.1, 100.5 and 1005.3 mg/kg dry soil . The results served for the determination of the No Observed Effect Concentration (NOEC) based on the biomass deviation throughout the test period.

The definitive test was first performed as a limit test atinitial concentrations 1.6, 3.0, 5.2, 9.6, 17.2, 30.8, 55.6 and 100.0 mg/kg dry soil .

The definitive test was thus reproduced as a full definitive test using four replicate units each containing 10 worms for each of eight test item treatments between 1.6 and 100 mg/Kg dry soil, and 8 replicate units for each the water control and the solvent control, as recommended in the guideline for a combines ECx and NOEC approach. The test item concentrations were not checked for the three lowest test item treatments because the nominal values were below the quantification level for HPLC determination of cypermethrin.

The adults were maintained in the artificial soil substrate for 4 weeks. Then, the observations consisted in percent mortality and mean weight of the survivals.

The adults were discarded and the test units were maintained in the climatic chamber for 4 additional weeks. At the end of the period, the number of juveniles was assessed.

One additional set of units were performed and conducted as the test system but without earthworm (abiotic units). They were used for the assessment of the test item concentration during the adult exposure period. The test item concentrations were not checked for the three lowest test item treatments because the nominal values were below the quantification level for HPLC determination of cypermethrin.

The measured concentrations for cypermethrin concentrations in the un-populated units represented more than 89 % of the nominal values over the first 28 days of the test (See Table 4.2.4.2.2.1) i.e. over the adult exposure period. Thus the test item treatments were not further adjusted in the populated unit.

Table 4.2.4.2.2 .1Measured concentration of cypermethrin in the un-populated units

Period of assessement		Nominal values, mg/kg				
		9.6	17.2	30.8	55.7	100.1
Day 0	Measured mg/kg	9.04	16.59	29.46	51.64	91.26
	% recovery	94.1 %	96.3 %	95.6 %	92.8 %	91.2 %
Day 7	Measured mg/kg	10.06	16.62	31.93	57.90	99.81
	% recovery	104.6 %	96.5 %	103.6 %	104.0 %	99.7 %
Day 13	Measured mg/kg	9.36	16.34	27.47	51.01	100.62
	% recovery	97.4 %	94.9 %	89.1 %	91.7 %	100.5 %
Day 21	Measured mg/kg	9.64	17.09	29.06	54.28	99.46
	% recovery	100.3 %	99.2 %	94.3 %	97.5 %	99.4 %
Day 28	Measured mg/kg	10.10	17.55	31.21	59.08	108.09
	% recovery	105.1 %	101.9 %	101.2 %	106.2 %	108.0 %

At the end of the test, the measured concentrations in the populated unit represented 62-77% of the nominal values.

Table 4.2.4.2.2.2 Measured concentration of cypermethrin in the populated units at the end of the test

	Nominal values, mg/kg				
	9.6	17.2	30.8	55.7	100.1
Replicate 1	6.23	11.20	20.87	36.99	78.79
Replicate 2	6.36	11.00	21.06	38.09	73.13
Replicate 3	5.74	10.96	20.38	36.73	75.17
Replicate 4	5.56	11.00	20.22	39.21	81.60
Mean mg/kg	5.97	11.04	20.63	37.76	77.17
Mean % recovery	62.2 %	64.1 %	66.9 %	67.8 %	77.1 %

No mortality was observed for the water controls, solvent controls or any of the test item treatments.

Biomass Changes

For both the water controls and the solvent, the final biomass represented more than twice the initial biomass.

That was also the case for every test item treatments up to and including 30.8 mg/Kg: F-variance analysis at the 5% confidence level showed that mean gain of biomass was similar to that of the water controls.

At both 55.6 and 100.0 mg/Kg mean gain of biomass was significantly reduced (5% confidence level).

For the reference item treatments, the gain of biomass was considered as similar to the controls at 1 mg/Kg dry soil, but significantly reduced at 2 and 5 mg/Kg dry soil.

Production of juveniles

In the control group, the mean number of juveniles was 99.5 per unit with a relative standard deviation of 15.5%.

The solvent controls gave similar values: mean reproductive performance was 98.5 juveniles per unit with a relative standard deviation of 18.7 %.

The water controls and the solvent controls were pooled to improve the sensitivity of the statistical analysis. Mean value for juveniles/unit was 99.0 with a relative standard deviation of 8.4 %.

F-variance analysis at a 5% confidence level showed that the reproductive performance was similar to the controls for every test item treatment up to and including 5.2 mg/Kg. For every higher test item treatment, the production of juveniles was significantly reduced (5% confidence level).

NOEC (reproduction) = 5.2 mg/Kg dry soil

Since no analytical measurement is available for the NOEC and the recovery is lower for the lower test concentrations, we propose to use the mean recovery of 62 % found for the nominal concentration 9.6 mg/kg to estimate the mean exposure concentration. This result in a NOEC of 4 mg/kg dw (geometric mean of 5.2 mg/kg and 3,2 mg/kg).

Conclusion: NOEC mortality is >100mg/Kg. The NOEC biomass is 30.8mg/Kg and the NOEC reproduction is 4.0 mg/Kg dw or 4.52 mg/Kg ww. The EC_{50} reproduction is 22.5mg/Kg

4.2.6.3 Toxicity of cypermthrin on terrestrial microbial fauna.

Servajean E (2005) has studied the effects of cypermethrin on the miniralization of nitrogen in soil.The study was performed in compliance with the OECD standard methods n°216 as adopted in January 2000.

Guideline / Test method	Species / process	Endpoint / Type of test	Exposure		Results			Reference
			Soil type	duration	NOEC	LOEC	EC/LC_{50}	
OECD 216 (2000)	*Nitrification*	*% effect*	*Loamy sand 1.77 % OM*	*28d*	*52.0 mg/Kg (dry soil)*	*93.6 mg/Kg (dry soil)*	/	Servajean E (2005)

A range-finding test was performed using one replicate unit for each of the five treatment concentrations between 0.1 and 1000mg/Kg dry soil. The final test was performed using three replicates bulk samples of treated soil for each of the five treatment concentration between 8.9 and 93.6mg/Kg dry soil so that the NOEC was encircled. Concentration above were difficult to test due to the properties of cypermethrin wich rend the soil sticky and nearly impossible to homogenate. The concentration of the treatment solutions were confirmed by HPLC analisys. Even distribution of the test substances was controlled soons after the application and percentage of recovery was calculated. After 0, 7, and 28 day of incubation, samples were extracted with aqueous solvent and the quantities of nitrates in the extracts were determined. These quantities were compared to that of a water control, of a solvent control and of a toxic reference (Fumical, 508 g/L metam sodium). Percentage of deviation compaired to the water control was calculated with respect to each test concentration, respectively.

The nitrate concentration was the same level at test initiation for every treatment and replicates. On day 7, nitrate concentration was reduced by 1 to 2 mg/Kg in the water control and in the solvent control as well. That was also the case for the 8.9 mg/Kg cypermthrine treated soil. In all other cases, including the

toxic reference, the decrease was much more accentuated than that in the water control soil. The deviation to the water control was significant for the 93.6 mg/Kg cypermthrin treated group.

On day 28, the production of nitrate ranged between 98.1% and 105.8% that of the water control group for every treated groups ranging for treatment concentration ranging from 8.9 to 52.0mg/Kg.

In the 93.6mg/Kg, the production of nitrate was only 78.9% that of the water control group wich was significantly different from the water control.

The EC_{50} value was not encircled.

In conclusion, the LOEC was calculated to 93.6 mg/Kg dry soil and the NOEC was equal to 52.0 mg/Kg dry soil.

4.2.6.4 Toxicity to other soil organisms.

In a field trial (GLP compliant) to determine the effects of Cyperkill 10EC (100g/L cypermethrin; 250 ml/ha) on the non target arthropod fauna (Carabidae; Staphylinidae; Linyphiidae; Collembolla; Diptera; Braconidae/ Ichneumonidae+ Aphidius Sp.; Gamebird-chick food) of a winter wheat crop, following two applications during May and June, Halsall N.; 2003, concluded that no adverse effect occured on non- targets arthropods populations. Effects on Carabid and Staphilinid were only transcient and both populations recovered within the same crop growing season.

A second study on the effects of cyperkill 10EC (100g/L cypermethrin; 250ml/ha) on the invertabrate soil meso-and macrofauna following two sequential application of the product 19 days apart, performed by Vinall S. ,2003, concluded that no adverse effect compared to control (Carbendazim) occurs on the number of invertebrates present in the surface layer of soil.

In a third study, the effect of two sequential applications 19 apart of cyperkill 10EC (100g/L cypermthrin; 250ml/ha) on straw decomposition were assessed in a field trial by Vinall S. The autor concluded that no effect on the decomposition of the straw compared to the blanc and controls (carbendazim) occurs.

Conclusion: The two first studies above support the fact that sequential application of cypermethrin on soil as a moderate effect on soil organism which population recovers. The third study indicated that cypermethrin has no impat on organic matter decomposition. Due to the application patern these studies are not relevant for the assessment of cypermethrin as wood preservative.

4.2.6.5 Toxicity to other non-target terrestrial organisms (secondary poisoning)

No test to evaluate the bioaccumulation in soil of cypermethrin cis:trans/40:60 was provided. The applicant provides some justification base on the fish bioacculmulation test and based on the Log kow value. Cypermethrin cis:trans/40:60 is non-polar. Log Kow values ranged from 5.3-5.6, which indicates a high potential for bioaccumulation according to the TGD. However, an experimental BCF of 373+/-45 in fish indicates it has a lower bioaccumulation potential which is confirmed by the result of the QSAR EPIWIN which provide a value of 417 L/Kg. (The latest is used for the risk assessment). Nevertheless, this test ((Szeleczky, 1990, report 90-016 CYP/T133) contains some deviation wich might impair the accuracy of the results. For exemple, the duration was only 10 days in place of 28 days, and the

chemical was tested with a solvent to enhance the solubility. According to the TGD page 126-127 these parameters are of importance to correctly estimate the bioaccumulation potential of a chemical. Regarding the bioaccumulationby terrestrial organisms, Cypermethrin has a high Koc value which ranges from 80653 to 574360 (Covance report 1669/015), which indicate that the active would adhere to soil/sediment making it very difficult for organisms to uptake and accumulate it. Conversely, the active may also absorbe to biological surface such as skin wich may lead to toxic effects in higher organisms after biomagnification.

4.2.6.6 Acute toxicity of cypermethrin to birds

[redacted], has investigated the acute toxicity of cypermethrin *cis:trans*/40:60 to bird.

Cypermethrin technical active substance (purity 96.5%) was administered in the diet to 10-day old northern bobwhite quail (*Colinus virginianus*) at nominal doses of 0, 562, 1000, 1780, 3160 and 5620 ppm a.i. for five days according to OECD guideline 205 (10 birds of undetermined sex/treatment group).

Guideline/ Test method	Spp.	Endpoint /type of test	Exposure		Results (mg a.s./L) measured		Reference
			design	duration	LC50/EC50	NOEC	
OECD 205	Northern bobwhite quail	mortality		5 day	LC50 (5d) > *1376 mg a.s./Kg bw/d	NOEC (5d) = 1000 mg a.s./Kg feed.	[redacted]

Mortalities, health and clinical observations were recorded twice daily for seven days after the beginning of dosing, and once prior to euthanasia, on Day 8 of the test. Individual body weights were measured at Days 0 (test initiation), 5 and 8. Average food consumption was determined by measuring the change in weight of feed presented to the birds during the exposure period and the post-exposure period.

No mortality observed in any treatment group. No clinical signs of toxicity nor modification of appearance and behaviour at 562 and 1000 g/Kg food. Signs of toxicity (wing droop, ruffled appearance, lethargy, hyper excitability) were observed at 1780, 3160 and 5620 mg/Kg food.

Treatment-related effect in bodyweight was observed during the exposure period at 3160 and 5620 mg/Kg food.

Clinical signs/feed consumption: no apparent treatment-related effect during exposure period.

LC50 (5d) > 5620 mg a.s./Kg feed or > 1376 mg a.s./Kg bw/d, based on the mean body weight of 24.5 g and the food consumption of 6 g /day reported in the 5620 mg/Kg treatment group.

The no observed effect concentration (NOEC) was 1000 ppm a.i. (1000 mg a.s./Kg feed) based upon signs of toxicity in birds receiving the 1780 ppm a.i. test concentration.

Conclusion:

LC50 (5d) > 5620 mg a.s./Kg feed or > 1376 mg a.s./Kg bw/d, based on no mortality at the highest dose level

4.2.6.7 Long term toxicity to birds

[redacted] has evaluated the long-term effect of oral ingestion of cypermethrin *cis-trans*/46:60 on the reproduction of the northern Bobwhite quail. Cypermethrin technical active substance (96.5% purity) was administered in the diet to twenty-nine week old northern bobwhite quail (*Colinus virginianus*) at nominal doses of 0, 160, 400 and 1,000 ppm a.i. for 21 weeks.

Guideline/ Test method	Spp.	Endpoint /type of test	Exposure		Results (mg a.s./L) measured		Reference
			design	duration	LC50/EC50	NOEC	
OECD 206	Northern bobwhite quail	reproduction		21 weeks		NOEC (21 weeks) = 92.0 mg a.s./Kg bw/d	[redacted]

Body weights of the adults were recorded before dosing and at two week intervals up to eight weeks after dosing, and then at the end of the study. Food consumption per pen was recorded weekly.

Any eggs were collected daily and examined for cracking and eggshell thickness measured. Eggs were incubated and examined for embryo viability and survival and allowed to hatch.

Macroscopic examinations were recorded at *post-mortem* on birds which died during the study and on all surviving birds at the end of the exposure period.

No treatment-related mortalities occurred during the study. There were no treatment-related effects on behaviour or appearance of adult birds and no overt signs of toxicity were observed at any of the concentrations tested. It was concluded that there were no treatment-related effects on the body weight of adult birds and any small differences in feed consumption were not considered to be treatment-related.

There were no treatment-related effects upon reproductive performance at any of the concentrations tested and no treatment-related macroscopic abnormalities were observed in any birds examined at autopsy.

Conclusion:

The NOEC (21 weeks) = 1000 mg a.s./Kg feed or 92.0 mg a.s./Kg bw/d

4.2.6.8 Other: Endocrine disrupting activity

The US EPA has published a "WEIGHT OF EVIDENCE ANALYSIS OF POTENTIAL INTERACTION WITH THE ESTROGEN, ANDROGEN OR THYROID PATHWAYS, CHEMICAL: CYPERMETHRIN" (June 2015) within their Endocrine Disruptor Screening Program (EDSP).

For the WoE analysis several new studies were considered. The report concludes "Cypermethrin demonstrates **no convincing evidence** of potential interaction with the estrogen or thyroid pathways. However there appears to be a **potential for interaction** with the androgen (anti-androgenic) pathway in mammals and fish."(p. 35).

In november 2016 , the criteria for identification of endocrine disrupters are still under discussion for the biocide regulation. The entry into force is foreseen for 2017.

Therefore, eCA suggest to to consider the available studies at the renewal stage of cypermethrin for PT8 or PT18.

4.3 CONCLUSION OF EFFECTS ASSESSEMENT

Cypermethrin *cis:trans*/40:60 shows:

An acute LC_{50} (96h) of 2.83µg/L on fish;An acute effect to *Daphnia magna* with a 48h EC_{50}= 4.71µg/L;

No toxicity to algae at concentration up to the water solubility value and above;

Inhibition of the microbial repiration at effective concentration of 163 mg/L;

A chronic toxicity to fish with an overall NOEC of 0.463 µg/L;

Has a chronic toxicity to Daphnia magna, with a NOEC = 0.04µg/L;

Cypermethrin tends to bioaccumulate in organism with a typical bioaccumulation factor of 417 L/Kg;

An NOAEC of 0.05µg/L for the macrozoobenthos community;

An overall NOAEC of 1 µg/L for the phytoplankton and of 0.05µg/L for the periphyton;

An NOAEC of 1.0 µg/L for the macrophytes;

No clear phytotoxicity effect on higher plant;

A LC_{50} for earthworms > 100mg/Kg;

A NOEC reproduction for earthworms = 5.2mg/Kg dry soil;

An inhibition potential for nitrogen mineralization at concentration above 52.0 mg/Kg $_{dry\ soil}$;

No effect on organic matter decomposition;

Transcient effect on soil invertebrate other than worms with recovery of population;

No acute effect on birds at concentration above 1376 mg a.s./Kg bw/d;

No long term toxicity to bird at concentration up to 92.0 mg a.s./Kg bw/d;

4.4 PNEC CALCULATIONS

4.4.1 PNEC water

The results of the mesocosm sudy can not been directly used to derive the PNEC water. However, according to recent decision (02/2010) at technical metting level, if the most sensible organism identified in a mesocosm study has been tested by conventional test method a mesocosm study can be used as support to lower the assessment factor. In our case, the most sensitive organism identified in the mesocosm study was not tested within a conventional laboratory study. The lowest NOEC calculated is 0.04µg/L for daphnia. Therefore, using the AF of 10, the PNEC water is 0.004µg/L

PNECwater = 0.004 µg/L

4.4.2 PNEC sediment

No study allow for the derivation of a PNEC sed. The equilibrium partitioning method should be applied. Since the PNEC sed is derived from the PNEC water, no convertion dry/wet weight has to be done

Using the equilibrium partitioning method and a value of koc of 575000 to calculate $K_{susp\text{-}water}$ and an additional factor of 10

PNECsed= 0.005 mg/Kg$_{ww}$

4.4.3 PNEC in STP

The result of the microbial activity inhibition test is provided as an EC_{50}. According to the TGD, an assessment factor of 100 is applied to the 163mg/L EC_{50} to derive the PNEC.

PNECstp= 1.63mg/L

4.4.4 PNEC soil

Two acute tests on earthworms was provided, which both presented small deficiences. The study presenting the most conservative value for the earthworms was kept as key study with an LC_{50} of 100mg/Kg $_{dry\ soil}$. A reproduction study with earthworms provided a NOEC of 4.0mg/Kg $_{dry\ soil}$ based on measured concentration.

The field trial on miniralization of nitrogen in soil performed by Servajean, provided a NOEC of 52.0mg/Kg $_{ww}$

Additionnal studies on plant and non target arthropods indicated that cypermethrin has minor and transient effect on the evaluated organisms at PPP application rate (250ml/ha) following two sequential applications (14 or 19 days).

According to the TGD, an assessment factor of 50 can be used from the earthworms acute test, the chronic earthworms test and microbial inhibition test (two NOEC from two trophic levels). However, the result from the study on plant and the tests on non target arthropod which are non key studies does not normally allowed to further lower the AF. However the results of the tests enhance the confidence

on the overall picture of the toxicity of cypermthrin on soil and terrestrial organisms. The resulting Pnec is 0.08 mg/Kg soil from the chornic earthworm NOEC reproduction using and AF of 50.

PNECsoil = 0.08mg/Kg$_{soil\ dw}$

4.4.5 PNEC $_{oral, bird}$

The PNECoral for secondary poisoning of birds is derived by applying an assessment factor of 30 to the chronic NOEC of 1000 mg/Kg feed, resulting in a PNECoral,bird of 33.3 mg/Kg feed.

4.4.6 PNEC$_{oral,mammals}$

The PNECoral for secondary poisoning of mamal is derived by applying an assessment factor of 30 to the chronic rat study (McAusland, Butterworth, Hunt, 1978) NOEC of 5 mg/Kg bw/d, resulting in a PNECoral,mammals of 3.3 mg/Kg food.

5 HAZARD IDENTIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Thermal stability: Cypermethrin *cis:trans*/40:60 is stable at room temperature and under normal use conditions

Flammability: The neat active substance is not flammable

Flash point: The neat active substance is not flammable

Explosive properties: No potential for explosion is present.

Oxidising properties: Cypermethrin *cis:trans*/40:60 does not contain any reactive groups that are oxidising in nature.

Reactivity towards container material: the container material (polyethylene-lined steel) is compatible with cypermethrin.

Vapour pressure: The vapour pressure of the neat substance is very low at 6 x 10^{-7} Pa at 25°C

Conclusion:

Cypermethrin *cis:trans*/40:60 does not demonstrate any particular hazardous physico-chemical properties. It is a stable, non-flammable chemical, with a low vapour pressure.

6 REFERENCES

Please refer to the single list of reference (doc RefList_PT8)