Section A1 Applicant

Annex Point IIA1

1.1 Applicant

Name: Agriphar s.a.
Address: Rue de Renory, 26/1, B-4102 Ougrée, Belgium
Telephone: [redacted]
Fax number: [redacted]
E-mail address:
[redacted]

1.2 Manufacturer of Active Substance (if different)

Name: Agriphar s.a.
Address: Rue de Renory, 26 B-4102 Ougrée, Belgium
Telephone: [redacted]
Fax number: [redacted]
E-mail address:
[redacted]
Location of manufacturing plant: See confidential folder DocIIIA_1_confidential_1

1.3 Manufacturer of Product(s) (if different)

Name: Agriphar s.a.
Address: Rue de Renory, 26 B-4102 Ougrée, Belgium
Telephone: [redacted]
Fax number: [redacted]
E-mail address:
[redacted]

Section A2 Identity of Active Substance

Subsection (Annex Point)			Official use only
2.1	**Common name (IIA2.1)**	Cypermethrin cis:trans/40:60	
2.2	**Chemical name (IIA2.2)**	IUPAC nomenclature :(RS)-α-cyano-3-phenoxybenzyl-(1RS)-cis, trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate	
		CA nomenclature : cyano(3-phenoxyphenyl)methyl-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane carboxylate	
2.3	**Manufacturer´s development code number(s) (IIA2.3)**	None	
2.4	**CAS No and EC numbers (IIA2.4)**	Non-entry field	
2.4.1	**CAS-No**	52315-07-8 (cypermethrin)	
	[1R-(1α(S*),3α)]	65731-84-2	
	[1S-(1α(R*),3α)]	72204-43-4	
	[1R-(1α(R*),3α)]	65731-83-1	
	[1S-(1α(S*),3α)]	72204-44-5	
	[1R-(1α(S*),3β)]	65732-07-2	
	[1S-(1α(R*),3β)]	83860-31-5	
	[1R-(1α(R*),3β)]	66841-24-5	
	[1S-(1α(S*),3β)]	83860-32-6	
2.4.2	**EC-No**	257-842-9 (cypermethrin)	
	[1R-(1α(S*),3α)]	265-898-0	
	[1S-(1α(R*),3α)]	276-457-7	
	[1R-(1α(R*),3α)]	265-897-5	
	[1S-(1α(S*),3α)]	276-458-2	
	[1R-(1α(S*),3β)]	265-899-6	
	[1S-(1α(R*),3β)]	281-086-9	
	[1R-(1α(R*),3β)]	266-492-6	
	[1S-(1α(S*),3β)]	281-087-4	
2.4.3	**Other**	CIPAC no. 332	
2.5	**Molecular and structural formula, molecular mass**		

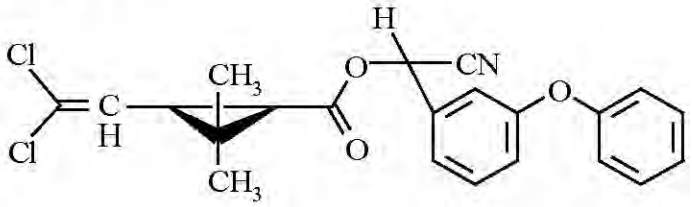
Section A2		Identity of Active Substance			
	(IIA2.5)				
2.5.1	**Molecular formula**	$C_{22}H_{19}Cl_2NO_3$			
2.5.2	**Structural formula**				
2.5.3	**Molecular mass**	416.3			
2.6	**Method of manufacture of the active substance (IIA2.1)**	See confidential information in Appendix to Document IIIA			
2.7	**Specification of the purity of the active substance, as appropriate (IIA2.7)**	g/kg 920	g/l	% w/w 92	% v/v
2.8	**Identity of impurities and additives, as appropriate (IIA2.8)**	See confidential information in Appendix to Document IIIA			
2.8.1	**Isomeric composition**	Cypermethrin cis:trans isomer ratio 40(±5) :60(±5). 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 A2_1and A2_2 and Fig. A2_1below.			
2.9	**The origin of the natural active substance or the precursor(s) of the active substance (IIA2.9)**	Not applicable			

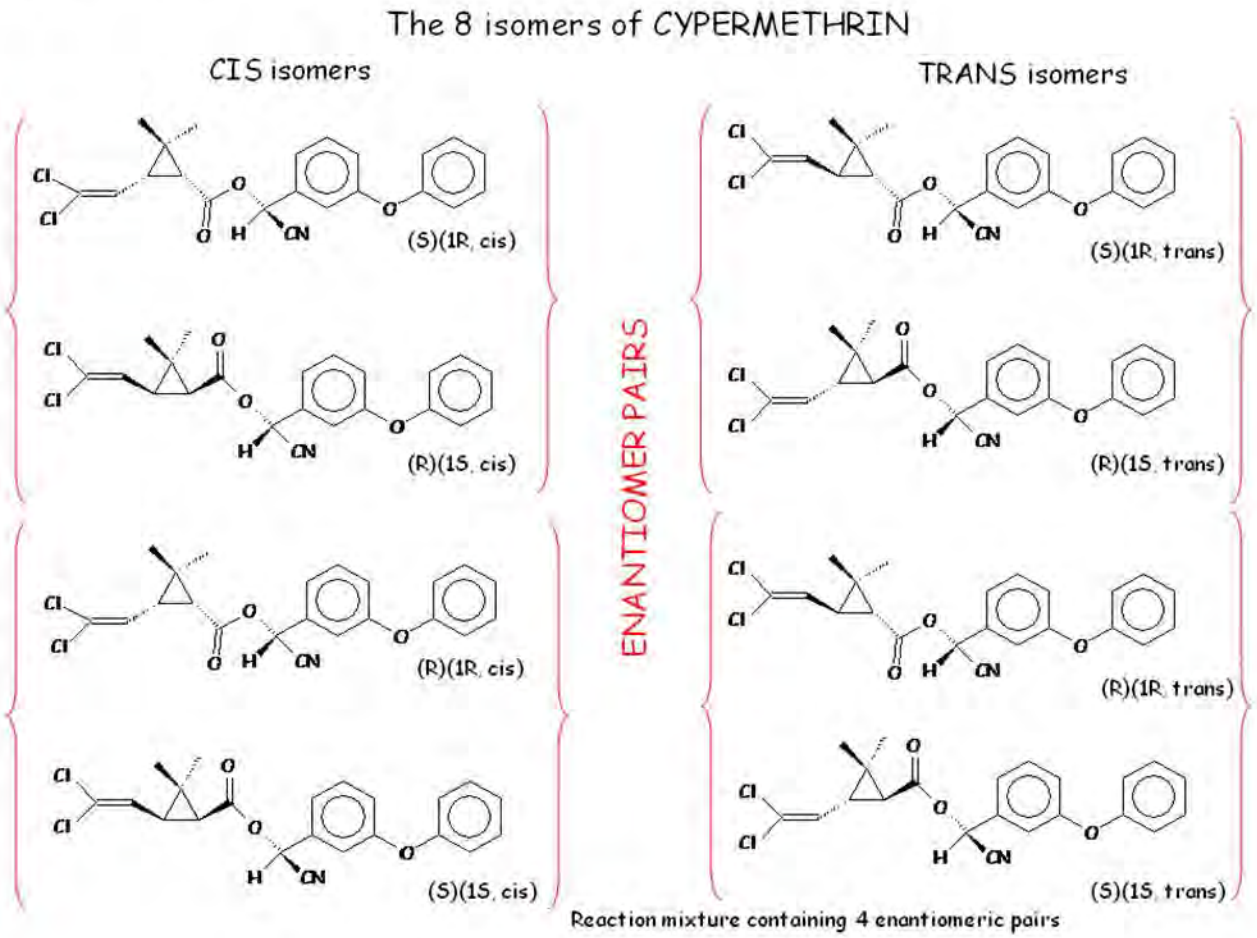
Table A2_1: Overview of the eight isomers of cypermethrin

	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 A2_2: 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%

Fig. A2-1: 8 Isomers of Cypermethrin



Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Janvier 2010*
Materials and methods	*Identification of the active substance.*
Conclusion	*The applicant's version is adopted.*
Reliability	*This part of the study is in compliance with agreed protocols.*
Acceptability	*Acceptable*
Remarks	*Document completed after quality check by Com to detailed the isomeric composition according to similar request made to other pyrethroïd's dossiers.*
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A2.10	**Exposure data in conformity with Annex VIIA to Council Directive 92/32/EEC (OJ No L, 05.06.1992, p. 1) amending Council Directive 67/548/EEC**
Annex Point IIA2.10	

Subsection		**Official use only**
2.10.1 Human exposure towards active substance		
2.10.1.1 Production		
i) Description of process	See point A2.6 in Confidential Annex	
ii) Workplace description	Emission from the reaction vessel is through a thermal oxidiser which operates at approximately 1000°C and flamelessly incinerates any volatile material. All raw materials are loaded to the reactor from storage tanks and the storage tanks are filled from road tankers or drums. Any operator exposure is assessed through COSHH (Control of Substances Hazardous to Health Regulations) and the appropriate PPE is worn. When sampling or drumming cypermethrin, an impervious plastic suit, gauntlets, boots, goggles, visor, vapour-mask and helmet are worn. Waste water is treated with formaldehyde to remove residual sodium cyanide and with sodium hydroxide and heated to breakdown any residual cypermethrin. The waste water is then sent to an approved waste disposal company. The plant is washed down with solvents and water and these are disposed of by incineration. Most of the raw materials are delivered in bulk so there are no issues with packaging waste. The acid chloride drums are recycled. Aqueous effluent in the storage tank is treated to remove residual sodium cyanide and traces of Cypermethrin. The aqueous waste is sent to an approved contractor for disposal. Exposure to raw materials and product is controlled through the use of appropriate personal protective equipment.	
iii) Inhalation exposure	No measured/monitoring data is available. Pure cypermethrin has a melting point of 41-47°C. Technical grade cypermethrin is a viscous liquid / semi-solid. During sampling and drumming there is a very low probability that inhalable airborne particles are formed. Inhalation of vaporised cypermethrin can only occur in the workplace when sampling and drumming neat cypermethrin or during cleaning and maintenance of plant equipment. The process is a closed system from manufacture to drumming. The EASE model (TGD for Risk Assessment) is used to assess inhalation exposure. The vapour pressure of Cypermethrin is 6×10^{-7} Pa at 25°C. Also during manufacture it has a low tendency to become airborne; the process is an enclosed system. Since the vapour pressure is less than 0.001kPa it is classed as having a very low volatility. Being a liquid with very low volatility and no likelihood of aerosol formation, it can be determined that it has a very low tendency to become airborne and is	

Section A2.10 **Annex Point IIA2.10**	**Exposure data in conformity with Annex VIIA to Council Directive 92/32/EEC (OJ No L, 05.06.1992, p. 1) amending Council Directive 67/548/EEC**

	therefore assigned an inhalation exposure of 0-0.1ppm. (see EASE flowchart Annex III of Document II).
iv) Dermal exposure	Direct dermal contact with cypermethrin cis:trans/40:60 during industrial manufacture is not foreseen due to the fact that this is a closed system and PPE is always worn by the operators. Using the EASE model it can be determined that in the enclosed system used, dermal exposure will be very low. It is therefore not considered further.
2.10.1.2 Production of the formulated product	
i) Description of process	Confidential (see IIIA2.6)
ii) Workplace description	This product is produced batch-wise in an enclosed system from manufacture to drumming. The amount of cypermethrin 40:60 cis/trans to be used for the production of the ME formulation is not currently known as this is a new product. However, the amount of cypermethrin used in biocides as a whole (PT8 and PT18) is expected to be much less than that used in agro-chemicals and will be order-driven. The active substance and the product are only handled by industrial operators with adequate training and protective equipment (gloves, boots, Tyvec overalls and mask with organic-vapour filter).
iii) Inhalation exposure	Pure cypermethrin has a melting point of 41-47°C. Technical grade cypermethrin is a viscous liquid / semi-solid. Before being used the drums are warmed in an oven to ensure homogeneity and aid transfer. There is a very low probability that inhalable airborne particles are formed. The possibility of particle or aerosol formation is zero once dissolution of the cypermethrin has taken place. Cypermethrin is transferred to the reactor, via an open manway, using a pneumatic pump from the drum. Therefore inhalation of vaporised cypermethrin could only occur in the workplace when open containers of neat cypermethrin are handled, during the transfer to the vessel or during cleaning and maintenance of equipment. The concentration in air is limited by vapour pressure and can be calculated from the following equation: $W = (P*V*M) / (R*T)$ Where W is the amount of substance in $1m^3$ air (g) P is the vapour pressure (6×10^{-7} Pa) V is the volume of air ($1m^3$) M is the molecular weight (416) R is the gas constant (8.314 J/mol/K)

Section A2.10	**Exposure data in conformity with Annex VIIA to Council Directive 92/32/EEC (OJ No L, 05.06.1992, p. 1) amending Council Directive 67/548/EEC**
Annex Point IIA2.10	

T is the temperature (298K)

Using the values listed above, the saturation concentration is calculated to be 1.00 x 10^{-7} g/m^3 (worst case). Taking into account that the production takes place in a ventilated work area, the concentration is reduced to 1% of the saturation concentration, i.e. 1.00 x 10^{-9} g.m^3 (normal use). Taking also into account an inhalation rate of 1.25m^3/h, a work day of 8 hours and an adult of 60kg, this would lead to an inhalation exposure of 1.67 x 10^{-5} mg a.i./kg bw/day (worst case) and 1.67 x 10^{-7} mg a.i. /kg bw/day (normal use).

iv) Dermal exposure

Direct dermal contact with cypermethrin 40:60 is not foreseen. However, incidental contact is possible during transfer of the substance to the mixing vessel and during cleaning and disposal of the containers. Hands could be incidentally exposed, when the gloves used are contaminated on the inside. In the absence of other guidance the indicative exposure values are taken from the model 1 for dipping application described in the TNsG on human exposure. This model gives a worse-case value of 25.7 mg/min for exposure of hands inside gloves. Using a general exposure calculator (see Doc II, annex IV) and assuming that the duration of dermal exposure is 15 min/day, the dermal exposure is estimated to be 385.5mg of formulated product/day.

The highest exposure is during the dilution step i.e. during the production of the formulation to produce the 10 g/l ME, which contains 1% cypermethrin. Dermal exposure is calculated assuming a worker of 60kg bodyweight. In a tier 1 approach, dermal penetration is assumed to be 100%. The tier 2 approach considers a dermal penetration of 13%, as shown in an in-vitro skin penetration study (see Doc IIIA6.2-02). Using the general exposure calculator (Doc IIA, Annex IV):

Worst case dermal penetration of 100%: 385.5 x 0.01/60 = 0.064mg a.i./kg bw/day.

Reasonable worst case, dermal penetration 13%: 0.064 x 0.13 = 0.0083mg a.i./kg bw/day.

2.10.1.3 Intended use(s)

1. Industrial Users

i) Description of application process

Cypermethrin is used as an insecticide active ingredient in wood preservative products mainly as a preventative treatment. Such treatments are carried out in industrial premises, using either dipping or vacuum-pressure impregnation of timber.

Industrial spraying can also be carried out, using enclosed spray cabinets (e.g. pre-treatment of window frames).

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ii) Workplace description	Vacuum-pressure impregnation For vacuum-pressure, the treatment takes place in an entirely closed system (sealed vessel). The process is estimated to take around 540 minutes with approximately 3 cycles per 8 hour working day. Dipping For dipping, industrial scale plants will employ a high degree of automation to minimise contact with the treatment solution and the treated wood. Dipping is done mechanically and is operated from a control panel, with wood loaded into a cradle/frame before being lowered into solution. In most plants wood is loaded and unloaded using forklift trucks. In a large-scale facility, up to 20 tasks per day i.e. 3 minutes per task which give a total duration of 60 minutes Spraying Industrial spray cabinets are used to spray the treatment solution directly onto wood, typically used for window and door frames. It is assumed that up to 4 cycles per day can be performed. A closed system is used to minimise any contact with the spray solution. Full PPE (gloves, overalls, goggles and vapour mask) will be worn for each type of industrial treatment.
iii) Inhalation exposure	See Document II-B of dossier
iv) Dermal exposure	See Document II-B of dossier

2. Professional Users

i) Description of application process	See Document II-B of dossier.
ii) Workplace description	See Document II-B of dossier.
iii) Inhalation exposure	See Document II-B of dossier.
iv) Dermal exposure	See Document II-B of dossier.

3. Non-Professional Users

i) Description of application process	See Document II-B of dossier.
ii) Workplace description	See Document II-B of dossier.

Section A2.10 **Annex Point IIA2.10**	**Exposure data in conformity with Annex VIIA to Council Directive 92/32/EEC (OJ No L, 05.06.1992, p. 1) amending Council Directive 67/548/EEC**

iii) Inhalation exposure	See Document II-B of dossier.
iv) Dermal exposure	See Document II-B of dossier.

4. Secondary exposure

i) Description of application process	See Document II-B of dossier.
ii) Workplace description	See Document II-B of dossier.
iii) Inhalation exposure	See Document II-B of dossier.
iv) Dermal exposure	See Document II-B of dossier.
v) oral exposure	See Document II-B of dossier.

2.10.2.1 Production

Estimated environmental exposure has been calculated using the EUSES model and is discussed fully in the risk assessment (Doc IIB). It is estimated that the annual production volume going in to PT8 is approximately 75 tonnes.

In the absence of any monitoring data, the following PEC values were calculated using the EUSES 2.0.3. model:

Compartment	Regional PEC (mg/l)	Continental PEC (mg/l)
Surface water (dissolved)	4.06×10^{-8}	3.15×10^{-14}
Surface water (total)	4.09×10^{-8}	3.17×10^{-14}
Sediment	7.05×10^{-6}	5.46×10^{-12}
Agricultural soil (total)	9.95×10^{-8}	2.83×10^{-12}
Pore water of agricultural soil	1.26×10^{-9}	3.57×10^{-14}
Air	2.61×10^{-12}	1.45×10^{-14}

This can be considered an overestimate as cypermethrin is manufactured in a closed system in a strictly controlled plant. Any residual cypermethrin is disposed of by specialist contractors with no environmental release to any compartment.

The predicted environmental exposure for the formulation step will be similar to that of the active substance, as the annual amount of

Section A2.10 **Annex Point IIA2.10**	**Exposure data in conformity with Annex VIIA to Council Directive 92/32/EEC (OJ No L, 05.06.1992, p. 1) amending Council Directive 67/548/EEC**

active substance being used in PT8 formulations should be the same. Again the process is carried out under strictly controlled conditions in a closed system, therefore the PEC values will be no worse that those given above.

2.10.2.2 Intended use(s)

The potential environmental exposure arising from vacuum-pressure and dipping treatments has been calculated using USES 4.02 (see Document IIB).

Affected compartment(s):	
water	Yes, taken into account in EUSES 4.02
sediment	Yes, taken into account in EUSES 4.02
air	Yes, taken into account in EUSES 4.02
soil	Yes, taken into account in EUSES 4.02
Predicted concentration in the affected compartment(s)	See Document IIB for full details of the PEC calculations. The following values were calculated for vacuum-pressure and dipping.

water

	Local PEC (mg/L)
Vacuum-pressure treatment	
Surface water during emission episode	1.65×10^{-6}
Annual average in surface water	1.13×10^{-6}
Pore water of agricultural soil	3.42×10^{-8}
Pore water of grassland	1.37×10^{-8}
Groundwater under agricultural soil	3.42×10^{-5}
Dipping	
Surface water during emission episode	2.99×10^{-6}
Annual average in surface water	4.1×10^{-7}
Pore water of agricultural soil	1×10^{-7}
Pore water of grassland	4.01×10^{-8}
Groundwater under agricultural soil	1×10^{-4}

sediment

	Local PEC (mg/L)
Vacuum-pressure treatment	
Sediment during emission episode	9.32×10^{-3}
Dipping	
Sediment during emission episode	0.0169

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air

	Local PEC (mg/L)
Vacuum-pressure treatment	
Annual average in air	6.6×10^{-13}
Dipping	
Annual average in air	2.4×10^{-13}

soil

	Local PEC (mg/L)
Vacuum-pressure treatment	
Agricultural soil (total) averaged over 30 days	6.94×10^{-4}
Agricultural soil (total) averaged over 180 days	3.19×10^{-4}
Grassland (total) averaged over 180 days	1.28×10^{-4}
Dipping	
Agricultural soil (total) averaged over 30 days	1.48×10^{-3}
Agricultural soil (total) averaged over 180 days	9.33×10^{-4}
Grassland (total) averaged over 180 days	3.73×10^{-4}

Section A2.10 **Annex Point IIA2.10**	**Exposure data in conformity with Annex VIIA to Council Directive 92/32/EEC (OJ No L, 05.06.1992, p. 1) amending Council Directive 67/548/EEC**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	**November 2007 for human exposure**
Materials and methods	Applicants version is acceptable
Conclusion	Adopt applicant's version including revised version for some parts
Reliability	
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*

Section A3	Physical and Chemical Properties of the Active Substance							
Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.1 Melting point, boiling point, relative density (IIA3.1)								
3.1.1 Melting point	EEC A1 (DSC) OECD 102	Purified a.s., 98.3% w/w (cis:trans 40:60) Batch no. AH902	Melting endotherm : onset 41.2°C, peak 47.3°C	Acceptable	Yes	1	Bates, 2002a	
3.1.2 Boiling point	EEC A2 (DSC)	Purified a.s., 98.3% w/w (cis:trans 40:60) Batch no. AH902	Boiling did not occur, but a decomposition exotherm was observed, starting at ca. 200°C	Acceptable	Yes	1	Bates, 2002a	
3.1.3 Bulk density/ relative density	EEC A3 (gas comparison pyknometer method)	Purified a.s., 98.3% w/w (cis:trans 40:60) Batch no. AH902	D_4^{20} = 1.303	Acceptable	Yes	1	Bates, 2002a	
3.2 Vapour pressure (IIA3.2)								
Vapour pressure 1	OECD 104	Purified a.s., 99.3% (cis:trans/40:60) Batch no. AH1058	**temperature:** 25°C **result:** 6×10^{-7} Pa	Acceptable	Yes	1	Sydney, 2005a	

Section A3	Physical and Chemical Properties of the Active Substance							
Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.2.1 Henry's Law Constant (Pt. I-A3.2)	Calculation	Purified a.s., 98.3% w/w (cis:trans 40:60) Batch no. AH902	H = 0.024 $Pa.m^3.mol^{-1}$ at 20°C calculated using : Vapour pressure at 20°C = 2.3×10^{-7} Pa Water solubility at 20°C 4 µg/L = 9.6×10^{-6} mol/m^3	Acceptable	Yes	1	Bates, 2002a	
3.3 Appearance (IIA3.3)								
3.3.1 Physical state	Laboratory observation	Pure a.s. 99.9% (cis:trans 40:60) Batch no. AS85/04 Technical a.s. 93.05% (cis:trans 40:60) Batch SL25163S63	Powder Viscous liquid	Acceptable	Yes	1	Bates, 2005	
3.3.2 Colour	Laboratory observation	Pure a.s. 99.9% (cis:trans 40:60) Batch no. AS85/04 Technical a.s. (93.05%) Batch SL25163S63	White Amber	Acceptable	Yes	1	Bates 2005	
3.3.3 Odour	Laboratory and manufacturing plant observations	Purified a.s. (≥ 98% pure) Technical a.s. as manufactured (≥ 92% pure)	Mild, chemical odour Mild, chemical odour	Acceptable	No	2	No report	

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Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.4 Absorption spectra (IIA3.4)								
UV/VIS	Spectra recorded from 200-800 NM	Purified a.s., 99.4% pure (cis:trans 40:60) Batch AS85/02	Absorption spectra similar under unadjusted and acidified conditions. Two absorbance maxima at 202-204 and 278nm. Change in spectra under alkaline conditions with a shift of absorbance maxima to 220 and 307 nm (bathochromic effect). in methanol, unadjusted pH : absorption maxima : 204 nm, ε = 43217 L.mol-1.cm-1 278 nm, ε = 2368 L.mol-1.cm-1 absorption at λ > 290 nm : 290 nm, ε = 839 L.mol-1.cm-1 295 nm, ε = 411 L.mol-1.cm-1 304 nm, ε = 332 L.mol-1.cm-1 314 nm, ε = 316 L.mol-1.cm-1	Acceptable	Yes	1	Greenwood, 2004	X

Section A3	Physical and Chemical Properties of the Active Substance							
Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
IR	KBr recorded over scan range 4000-500 cm^{-1}	Purified a.s., 99.3% (cis:trans/40:60) Batch no. AH1058	Spectrum consistent with assigned structure of cypermethrin	Acceptable	Yes	1	Sydney, 2005a	
NMR	Proton NMR	Purified a.s., 99.3% (cis:trans/40:60) Batch no. AH1058	Spectrum consistent with assigned structure of cypermethrin	Acceptable	Yes	1	Sydney, 2005a	
MS	GC / Electron Impact (EI) mass spectrum	Purified a.s., 99.3% (cis:trans/40:60) Batch no. AH1058	Spectrum consistent with assigned structure of cypermethrin	Acceptable	Yes	1	Sydney, 2005a	
3.5 Solubility in water (IIA3.5)								
Water solubility	EEC A6 (column elution method + HPLC-UV)	Purified a.s. 99.5% (cis:trans 40:60) Batch AS85/00	**Result:*** Double distilled water (pH 6) : < 9 µg/L 1% acetonitrile in 0.02M phthalate buffer (pH 4) : < 9 µg/L **temperature:** 20°C	Acceptable. Result confirms literature data.	Yes	1	Bates, 2002a	
3.6 Dissociation constant (-)				Not applicable, product has very low water solubility				X
3.7 Solubility in organic solvents, including the effect of temperature on solubility (IIIA3.1)	OECD 105	Purified a.s., 99.3% (cis:trans/40:60) Batch no. AH1058	**result:** methanol 248 g/L Heptane 57 g/L **temperature:** 20°C	Acceptable	Yes	1	Sydney, 2005a	X

Section A3	Physical and Chemical Properties of the Active Substance							
Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.8 Stability in organic solvents used in b.p. and identity of relevant breakdown products (IIIA3.2)				Not applicable, refer to storage stability trial for the formulated product in DocIIIB				X
3.9 Partition coefficient n-octanol/water (IIA3.6)								
log Pow	EEC A8 (HPLC-method)	Purified a.s., 98.3% w/w (cis:trans 40:60) Batch no. AH902	Cypermethrin eluted as four discrete components with retention times corresponding to log P_{ow} values ranging from 5.3 to 5.6 (25°C; mobile phase methanol:water 75:25)	Acceptable	Yes	1	Bates, 2002a	
3.10 Thermal stability, identity of relevant breakdown products (IIA3.7)	OECD 113	Technical a.s. 93.05% (cis:trans 40:60) Batch SL25163S63	Thermally stable at room temperature with no decomposition or transformation below 150°C	Acceptable	Yes	1	Sydney, 2005b	
3.11 Flammability, including auto-flammability and identity of combustion products (IIA3.8)	EEC A15 (ASTM-E 659-78)	Technical a.s. 96.5% (cis:trans/40:60) Batch 2001060167	auto-ignition temperature = 400°C	Acceptable	Yes	1	Bates 2002b	
3.12 Flash-point (IIA3.9)	EEC A9 (closed cup equilibrium method)	Technical a.s. 96.5% (cis:trans/40:60) Batch 2001060167	no flash up to 110°C	Acceptable	Yes	1	Bates 2002b	

Section A3	Physical and Chemical Properties of the Active Substance							
Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.13 Surface tension (IIA3.10)				Not applicable, solubility <1 mg/L				X
3.14 Viscosity (-)	OECD 114	Technical a.s. 93.05% (cis:trans/40:60) Batch SL25163S63	**Result:** >40000 mPa.s **temperature:** 20°C **Result:** 1700 mPa.s **temperature:** 40°C	Acceptable	Yes	1	Sydney, 2005b	
3.15 Explosive properties (IIA3.11)	Assessment of chemical structure and thermodynamic properties according to EEC A14	Technical a.s. 96.5% (cis:trans/40:60) Batch 2001060167	Oxygen balance (-194.1%) is at the limit of the region where explosion potential exists. No auxoploses /plosophores are present in the structure. Decomposition exotherms are not sharp and enthalpy of each exotherm is below trigger value of 500 J/g. No potential for explosion is present	Acceptable	Yes	1	Bates 2002b	
3.16 Oxidizing properties (IIA3.12)	EPA OPPTS 830-6314	Technical a.s. 96.5% (cis:trans/40:60) Batch 2001060167	Assessed against zinc and potassium permanganate, and for chemical incompatibility with monoammonium phosphate and with water. No adverse reaction was observed in any test	Acceptable	Yes	1	Bates 2002b	

Section A3	Physical and Chemical Properties of the Active Substance							
Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.17 Reactivity towards container material (IIA3.13)	Information only	-	Cypermethrin is not corrosive to container materials and can be safely stored in HDPE, glass or aluminium bottles. Manufactured a.s. can be shipped in lacquer or poly-lined steel drums based on 2 year storage stability data.	Acceptable	-	2	None	X

* Other results from analytical report from study in environment part and in the Tomlin Pesticide Manual provide a value of 4µg/l. This latest has been used to calculate the H constant and in the risk assessment for environment

Evaluation by Competent Authorities	
	ABSORPTION SPECTRA (3.4)
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*July 2007*
Evaluation of applicant's justification	*The TGD mentions that absorption spectra must be submitted at relevant wavelengths. The European Food Safety Authority (EFSA) has revised Annex II and III to directive 91/414/EC and states that the wavelength of 290 nm must also be determined in the UV/VIS spectra. The value of the molar extinction coefficient ε at 290 nm is the trigger for the need of a direct phototransformation study (a study is only required if ε > 10 l mol^{-1} cm^{-1}).* *No result are given for the wavelength of 290 nm. Although, the applicant refers to a study of Greenwood (2004) in which the requested wavelengths have been studied.*
Conclusion	*The results for the other wavelengths should also been mentioned (cfr. the results given in Doc I, Appendix I)*
Acceptability	*Acceptable (with minor revision)*
Remarks	*None*
	COMMENTS FROM
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Evaluation by Competent Authorities	
	DISSOCIATION CONSTANT (3.6.)
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*July 2007*
Evaluation of applicant's justification	*No justification for non-submission of data is given. The "TGD on data requirement" mentions that a dissociation constant has not to be provided in case the water solubility cannot be measured. Since the water solubility is very low (< 9 µg/l), it can be agreed that the dissociation constant is not applicable.*
Conclusion	*The applicant's version should be changed in "not applicable, product has very low solubility in water" instead of "not applicable, product does not dissociate".*
Acceptability	*Acceptable (with minor revision)*
Remarks	*None*
	COMMENTS FROM ...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Evaluation by Competent Authorities
SOLUBILITY IN ORGANIC SOLVENTS (3.7)

	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*July 2007*
Evaluation of applicant's justification	*The applicant follows the guidelines as they are mentioned in TGD. Although, the EFSA has revised Annex II and III of directive 91/414/EC and states that the solubility must be determined at 15 to 25°C in different organic solvents (aliphatic hydrocarbon, aromatic hydrocarbon, halogenated hydrocarbon, alcohol, ketone and ester) and must be reported if less than 250 g/l. If for a particular active substance, one or more of these solvents are unsuitable, alternative solvents can be used instead. In such cases, choices made must be justified in terms of their structure and polarity.*
Conclusion	*It is advisable to consider more organic solvents*
Acceptability	*Acceptable (with revision)*
Remarks	*None*
	COMMENTS FROM IND
Date	*28 juillet 2008*
Results and discussion	*This test was performed specifically for our biocides dossier and therefore the appropriate TGD was used. Since this data has not been requested under 91/414/EC (see comments in the monograph), the solubility of cypermethrin in additional organic solvents is not available.*
Conclusion	
Reliability	
Acceptability	
Remarks	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*03 OCTOBER 2008*

Results and discussion	*I looked to find the comments in the monograph where is stated that no more solubility data of cypermethrin in additional organic solvents have to be given. I couldn't find it back. Can this information be provided by the manufacturer? At this moment I only know that based on the directive 91/414/EC we need more data than the ones that have been given now in the dossier.* *2.7. Solubility in organic solvents 91/414/EC* *The solubility of the active substances as manufactured in the following organic solvents at 15 to 25 °C must be determined and reported if less than 250 g/kg; the temperature applied must be specified:* *— Aliphatic hydrocarbon: preferably n-heptane,* *— Aromatic hydrocarbon: preferably xylene,* *— Halogenated hydrocarbon: preferably 1,2-dichlorethane,* *— Alcohol: preferably methanol or isopropyl alcohol,* *— Ketone: preferably acetone,* *— Ester: preferably ethyl acetate.* *If for a particular active substance, one or more of these solvents is unsuitable (e.g. reacts with test material), alternative solvents can be used instead. In such cases, choices made must be justified in terms of their structure and polarity*
	COMMENTS FROM IND
Date	*28 November 2008*
Results and discussion	We refer to Annex B, page 33 of the Cypermethrin monograph (point B2.1.13) for further information on the solubility in organic solvents. Agriphar has already provided data on solubility in an aliphatic hydrocarbon (heptane) and an alcohol (methanol). Solubility in an ester (ethyl acetate) is also available in the study conducted by Covance (Bates, 2002b, report no. 40/33-D2149 filed under Doc IV_A3.1. We believe this fulfils the requirements under Directive 98/8/EC where solubility in only two solvents is required. No further data was requested for Annex I inclusion of cypermethrin under 91/414/EC since the data submitted by Cyanamid (Grayson, 1975) is out of data protection.
Final Conclusion	**ACCEPTABLE BY THE RMS**
Reliability	
Acceptability	
Remarks	

Evaluation by Competent Authorities	
	STABILITY IN ORGANIC SOLVENTS USED IN BIOCIDAL PRODUCTS (3.8)
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*July 2007*
Evaluation of applicant's justification	*No justification for non-submission of data is given. Since the "TGD on data requirement" mentions that stability in organic solvents used in biocidal products must be stated only if the active substance as manufactured includes an organic substance, it can be agreed that stability in organic solvents is not applicable (no organic substance included in the formulation)*
Conclusion	*The applicant's version is adopted.*
Acceptability	*Acceptable*
Remarks	*None*
	COMMENTS FROM ...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Evaluation by Competent Authorities	
	SURFACE TENSION (3.13)
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 2007
Evaluation of applicant's justification	The TGD mentions that the surface tension should be measured using an aqueous solution of sufficient concentration such that any surface activity potential is expressed; i.e. at 90% or saturation to a maximum concentration of 1 g/l (where viscosity permits). Inconsistencies between the water solubility result and the solubility reported should be fully addressed. The EC method A.5 should be used.
Conclusion	The prescribed methodology has not been followed by the applicant
Acceptability	Not Acceptable
Remarks	None
	COMMENTS FROM IND
Date	*JUILLET 2008*
Results and discussion	It is our understanding that for substances with very low water solubility, determination of the surface tension is not considered necessary. EC method A5 (based on OECD 115) and the ISO method 304 both note that the testing of products with a water solubility of <1 mg/L is not applicable. It is not possible to achieve an aqueous solution of sufficient concentration such that any surface activity potential is expressed. This is also the opinion of our GLP test laboratory (Department Phytopharmacie, Gembloux, Belgium) who has also pointed out that if we use the method at 90% of water solubility of cypermethrin the result should be very close to that of pure water (since pure water is used to calibrate the tensiometer).
Conclusion	
Reliability	
Acceptability	
Remarks	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2008
Conclusion	This comment is acceptable.

Evaluation by Competent Authorities
REACTIVITY TOWARDS CONTAINER MATERIAL (A 3.17)

	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 2007
Evaluation of applicant's justification	Following the TGD, the information on reactivity towards container material should be obtained from experience in use and the chemical structure. No evidence is given by the applicant that the proposed results are based upon experiment and/or chemical structure.
Conclusion	Further information on the methodology must be submitted
Acceptability	Not acceptable
Remarks	None

	COMMENTS FROM ...
Date	Juillet 2008
Results and discussion	The comments made regarding the container material are based on general observations of material shipped to Agriphar. However, I have attached a summary of storage stability data generated by the manufacturer which shows that material stored in commercial packaging is stable for up to 50 months.
Conclusion	
Reliability	
Acceptability	
Remarks	

	Evaluation by Rapporteur Member State
	October 2008
	Although we don't see that the study performed was based on the interaction between the package and material we agree with this approach. OK Although, more information should be provided concerning the reference of these study results. At this moment only a table is presented. We don't know who did the study or where the table and data are taken from. As far as we can interprete the table, the study concerns 50 months. This means a storage stability of more than 4 years instead of the mentioned 2 years.
	COMMENTS FROM IND
Date	November 2008
Results and discussion	This data was obtained from the previous data owner Mitchell Cotts Chemicals and was generated using batches of Cypermethrin manufactured for veterinary use and stored under general warehouse conditions (not controlled temperature or relative humidity). The study is non-GLP and is submitted as additional evidence of stability of the active substance in the packaging. We therefore propose to keep the 2 year shelf-life claimed in the dossier.
Final conclusion	**ACCEPTABLE BY THE RMS**

Section IIIA.3.11 **Annex Point IIIA XIII 3.4**	**Flammability, including auto-flammability and identity of combustion products**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [√]** **Other justification [ ]**	
Detailed justification:	Flammability according to method EEC A12 was not performed as cypermethrin is not a solid or a gas. Instead the auto-ignition temperature was determined according to method A15. This method is more appropriate for substances with a low melting point (<50°C) according to the TNG. According to the TNGs, A13 pyrophoric properties is not mentioned in the core data set. Furthermore, this study is only applicable to substances which, in small amounts, ignite spontaneously in contact with air at room temperature. Based on the results of the test using method A.15 Auto-ignition temperature (=400°C), this is not applicable to cypermethrin.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A4 (4.2) Analytical Methods for Detection and Identification

Annex Point IIA 4.2 (a) Analytical method for the a.s. and residues thereof in soil

			Official use only
		1 REFERENCE	
1.1	**Reference**	Wimbush, J (2003); Cypermethrin: Validation of an analytical method for the determination and confirmation of residues in soil and sediment; Covance Laboratories Ltd, report no.40/039-D2149 (CYP/C70), 23 September 2003 (unpublished). Dates of experimental work: 12 April 2002 – 15 January 2003	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Chimac-Agriphar s.a.	
1.2.2			
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes Study undertaken in compliance with requirements of Directive 91/414/EEC of 15 July 1991 as amended by Commission Directive 96/46EC of 16 July 1996 and the guidance document on residue analytical methods SANCO/825/00 rev. 6 of 20 June 2000.	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	Soil: Extraction of Cypermethrin with acetone (3 times), followed by sonication and centrifugation. Rotary-evaporation of a combined aliquot and reconstitution in hexane. Sediment: Extraction of Cypermethrin with hexane/acetone (1:1 v/v) (3 times), followed by removal of water and acetone and concentration of the hexane extract to low volume.	
3.1.2	Cleanup	Co-extractives are removed by Florisil column chromatography and after evaporation the eluate is reconstituted in toluene.	
3.2	**Detection**		
3.2.1.	Separation method	Quantitative determination by capillary GC (DB-5MS, 30 m x 0.25 mm, 0.25 µm film thickness).	

Section A4 (4.2) **Analytical Methods for Detection and Identification**

Annex Point IIA 4.2 (a) **Analytical method for the a.s. and residues thereof in soil**

3.2.2	Detector	Soil: Mass Spectrometric detector using chemical ionisation in the negative ion mode (ions monitored: m/z 207, 209 and 211; ions for quantification : m/z 207 and 209) . Each diastereoisomer of Cypermethrin is measured individually and the total cypermethrin residue is calculated by summing the 4 individual diastereoisomers Sediment: Quantitative determination as for soil, except that quantification is based on a single ion (m/z 207); 2 additional ions (m/z 209 and 211) are monitored for qualitative confirmation
3.2.3	Standard(s)	Calibration is achieved using an internal reference marker (permethrin) which is added to the final extract prior to GC-MS analysis to compensate for any changes in detector response
3.2.4	Interfering substance(s)	None
3.3	**Linearity**	
3.3.1	Calibration range	0.005 to 1.0 mg/L total cypermethrin
3.3.2	Number of measurements	6 standard solutions of increasing cypermethrin concentration
3.3.3	Linearity	Correlation coefficient $r^2 > 0.999$
3.4	**Specificity: interfering substances**	No significant matrix interference (control values < 30% LOQ)
3.5	**Recovery rates at different levels**	See table A4.2(a)_1 (mz 207 ion) and table A4.2(a)_2 (mz 209 ion)
3.5.1	Relative standard deviation	See table A4.2(a)_1 (mz 207 ion) and table A4.2(a)_2 (mz 209 ion)
3.6	**Limit of determination**	Limit of Quantification (LOQ) = 0.05 mg/kg for soil and 0.5 µg/kg for sediment.
3.7	**Precision**	
3.7.1	Repeatability	Not determined
3.7.2	Independent laboratory validation	Not determined

Section A4 (4.2) Analytical Methods for Detection and Identification

Annex Point IIA 4.2 (a) **Analytical method for the a.s. and residues thereof in soil**

		4 APPLICANT'S SUMMARY AND CONCLUSION
4.1	**Materials and methods**	Gas Chromatography with Mass Spectrometric Detection (GC-MS), capable of measuring cypermethrin residues in soil and sediment. The method monitors 3 different ions and thereby confirms the presence of cypermethrin. Each stereoisomer is measured individually and the total cypermethrin residue calculated by summing the 4 individual stereoisomers.
4.2	**Conclusion**	Acceptable mean recover and precision at the lower validation levels confirmed that total cypermethrin is quantifiable at or above 0.05 mg/kg (0.5µg/kg for sediment).
4.2.1	Reliability	1
4.2.2	Deficiencies	No
		Study evaluated and accepted under Directive 91/414/EC.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March 2008
Materials and methods	Applicant's version is acceptable.
Conclusion	Applicant's version is Adopted.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A4.2(a)_1 Validation of methods CLE 0040/039-01V.SOIL and CLE 0040/039-02V.SED for residues in soil and sediment (m/z 207 ion)

Matrix	Analyte	Fortification level (mg/kg commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
soil	cis-I	0.0120	5	100 – 106	102	2.2
		0.1195	5	101 – 103	102	1.0
	trans-I	0.0165	5	104 – 109	107	1.8
		0.1650	5	103 – 104	104	0.5
	cis-II	0.0102	5	92 – 103	97	4.3
		0.1020	5	95 – 100	98	2.3
	trans-II	0.0114	5	94 – 101	97	2.7
		0.1135	5	93 – 96	95	1.5
	Cypermethrin	0.05	5	99 – 105	101	2.3
	(total isomers)	0.50	5	99 – 101	100	1.0
sediment	cis-I	0.1195 µg/kg	5	59 – 83	71	13.8
		1.195 µg/kg	5	78 – 86	82	3.7
	trans-I	0.165 µg/kg	5	55 – 73	**65**	11.0
		1.65 µg/kg	5	80 – 91	87	4.7
	cis-II	0.102 µg/kg	5	68 – 86	76	9.6
		1.02 µg/kg	5	95 – 107	103	4.7
	trans-II	0.1135 µg/kg	5	82 – 109	97	12.0
		1.135 µg/kg	5	110 – 136	**128**	8.3
	Cypermethrin	0.5 µg/kg	5	65 – 86	76	11.3
	(total isomers)	5.0 µg/kg	5	90 – 101	99	4.9

all recoveries were corrected for control values (< 30% of LOQ)

Table A4.2(a)_2 Validation of methods CLE 0040/039-01V.SOIL and CLE 0040/039-02V.SED for residues in soil and sediment (m/z 209 ion)

Matrix	Analyte	Fortification level (mg/kg commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
soil	cis-I	0.0120	5	99 – 105	101	2.3
		0.1195	5	100 – 103	101	1.3
	trans-I	0.0165	5	103 – 108	106	1.8
		0.1650	5	101 – 105	103	1.7
	cis-II	0.0102	5	93 – 101	96	3.6
		0.1020	5	93 – 100	97	2.8
	trans-II	0.0114	5	93 – 100	96	2.7
		0.1135	5	94 – 97	95	1.4
	Cypermethrin (total isomers)	0.05	5	98 – 104	101	2.4
		0.50	5	98 – 101	100	1.3
sediment	cis-I	0.1195 µg/kg	5	60 – 82	71	13.1
		1.195 µg/kg	5	79 – 86	82	3.5
	trans-I	0.165 µg/kg	5	55 – 73	**65**	10.6
		1.65 µg/kg	5	80 – 92	87	5.0
	cis-II	0.102 µg/kg	5	110 – 137	**123**	8.9
		1.02 µg/kg	5	153 – 175	**167**	5.2
	trans-II	0.1135 µg/kg	5	82 – 109	97	11.6
		1.135 µg/kg	5	111 – 136	**128**	7.8
	Cypermethrin (total isomers)	0.5 µg/kg	5	74 – 97	86	10.7
		5.0 µg/kg	5	102 – 116	**112**	4.9

all recoveries were corrected for control values (< 30% of LOQ)

Section A4 (4.2) (b) Analytical Methods for Detection and Identification

Annex Point IIA 4.2 (b) **Analytical method for the a.s. and residues thereof in air**

Official use only

		1 REFERENCE
1.1	**Reference**	Wimbush, J (2005); Cypermethrin cis:trans 40:60: Validation of an analytical method for the determination of residues in air; Covance Laboratories Ltd, report no.1669/016-D2149, 1 December 2005 (unpublished) Dates of experimental work: 29 March 2005 – 11 May 2005
1.2	**Data protection**	Yes
1.2.1	Data owner	Chimac-Agriphar s.a.
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes Study based on EU guidance documents on residue analytical methods SANCO/3029/99 rev.4 (11/07/00) and SANCO/825/00 rev.7 (17/03/04)
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Preliminary treatment**	
3.1.1	Enrichment	Residues of cypermethrin in air are trapped in suitable air sampling XAD-2 resin packed glass tubes then extracted with toluene in an ultrasonic bath.
3.1.2	Cleanup	Extracts are filtered through glass wool, combined, and the contents reduced to ca. 1ml using nitrogen convection at 40°C and then transferred to a volumetric flask and diluted to volume with toluene.
3.2	**Detection**	
3.2.1	Separation method	Detection, quantification and confirmation by gas chromatography using a Trace GC Ultra Gas Chromatograph, DB-5MS (30 m x 0.25 mm, 0.25 µm film thickness) at 250°C.
3.2.2	Detector	Mass spectrometric detection (GC/MS), with chemical ionisation in the negative ion mode. The method monitors for 3 different ions, therefore a separate confirmatory method is not required. Each diasterioisomer pair is measured separately and the total cypermethrin residue calculated by summing the concentration of the four diasterioisomer pairs.

Section A4 (4.2) (b)	**Analytical Methods for Detection and Identification**
Annex Point IIA 4.2 (b)	**Analytical method for the a.s. and residues thereof in air**

3.2.3	Standard(s)	Duplicate primary stock standard solutions of cypermethrin prepared in acetone. Concentrations of each diastereoisomer pair are calculated from the percentage of each, relative to total cypermethrin, as certified by the Certificate of Analysis. Individual concentrations are required to enable the four individual calibration lines to be inputted into the data system and for the recovery of each diastereoisomer pair to be evaluated. Analysis of final extracts should be carried out against at least six calibration standards. Final extracts containing concentrations greater than the top calibration point should be diluted with toluene, so that they fall within the calibration range.
3.2.4	Interfering substance(s)	None
3.3	**Linearity**	
3.3.1	Calibration range	In order to establish the linearity of response of the analytical chromatographic system to each diastereoisomer pair of cypermethrin, at least six standard solutions of increasing concentration were prepared. The lowest concentration was equivalent to less than 50% of a sample at the limit of quantification and the highest concentration was equivalent to greater than 110% of the highest level to be analysed. Response of the GC/MS system to each diastereoisomer pair of cypermethrin was non-linear (quadratic), for ion *m/z* 207 over the concentration range 0.01 to 0.3 µg/mL.
3.3.2	Number of measurements	Minimum of six calibration standards. Solutions were injected into the chromatograph in random order, and a concentration/response curve was prepared.
3.3.3	Linearity	Coefficient of determination (r^2) ≥ 0.98.
3.4	**Specifity: interfering substances**	Control traps were free from co-eluting components exceeding 30% of LOQ. Therefore, the analytical procedure was considered specific for the four diastereoisomers of cypermethrin in air
3.5	**Recovery rates at different levels**	Recovery of cypermethrin *cis:trans* 40:60, from traps fortified at 0.375 µg/m^3 (LOQ) and 3.75 µg/m^3 (10x LOQ), was determined in quintuplicate. In addition, control traps were extracted and analysed in duplicate. Where there was an apparent response from the control trap, to any of the cypermethrin diastereoisomer pairs, the equivalent concentration was subtracted from each of the validation levels, before calculation of the recovery values. Mean recoveries of total cypermethrin, at each fortification level and overall, were within the acceptable range of 70 to 110%. Acceptable mean recovery was obtained for each cypermethrin diastereoisomer pair (cis-I, trans-I, cis-II, trans-II) in air, with the exception of cis-II cypermethrin at 10x LOQ with a mean recovery of 111% (see table A4.2b_1)
3.5.1	Relative standard deviation	Relative Standard Deviation of the recovery measurements are presented in table A4.2b_2. Acceptable %RSD (≤ 20%.) was obtained at each fortification level and overall for total cypermethrin and for each diasterioisomer pair, with the exception of cis-I cypermethrin under ambient conditions (20.4% RSD).

Section A4 (4.2) (b) **Analytical Methods for Detection and Identification**

Annex Point IIA 4.2 (b) **Analytical method for the a.s. and residues thereof in air**

3.6	**Limit of determination**	Acceptable mean recovery (70 to 110%), and precision (≤ 20% RSD) was obtained for total cypermethrin, at each validation level, under both sets of sampling conditions. This confirmed the LOQ for total cypermethrin **in air** as 0.375 µg/m^3
3.7	**Precision**	
3.7.1	Repeatability	Repeatability of the method was expressed as the relative standard deviation (%RSD) of the recovery measurements as discussed in point 3.5.1.
3.7.2	Independent laboratory validation	Not performed

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	An analytical method for the determination and confirmation of cypermethrin in air, under two conditions of temperature and relative humidity, was developed and validated in accordance with SANCO guidelines. Control traps (XAD-2 resin packed glass tubes; 200/400 mg) were fortified with known amounts of cypermethrin cis:trans 40:60, at two levels in quintuplicate. The levels were equivalent to air concentrations of 0.375 µg/m^3 (LOQ) and 3.75 µg/m^3 (10x LOQ). The traps were then flushed with air, under ambient or elevated conditions of temperature and humidity, for six hours and analysed using the analytical procedure. Recovery of cypermethrin was determined for each sample and the results used to assess the validity of the analytical method .
4.2	**Conclusion**	Response of the GC/MS system to each cypermethrin diastereoisomer pair was non-linear (quadratic) over the concentration range 0.01 to 0.3 µg/mL (total cypermethrin), with coefficients of determination (r^2) ≥ 0.98. Control traps were free from co-eluting components exceeding 30% of LOQ. The analytical procedure was considered to be specific for the analysis of cypermethrin. Mean recovery for total cypermethrin was within the acceptable range of 70 to 110%, at each validation level, under each set of conditions. Precision of the method for total cypermethrin was also acceptable, with RSD ≤ 20% at each validation level. Acceptable mean recovery and precision confirmed the limit of quantification, for total cypermethrin in air, as 0.375 µg/m^3. Therefore, an analytical method was validated successfully for the determination and confirmation of total cypermethrin in air within the range 0.375 to 3.75 µg/m^3.
4.2.1	Reliability	1
4.2.2	Deficiencies	No

Section A4 (4.2) (b) Analytical Methods for Detection and Identification

Annex Point IIA 4.2 (b) **Analytical method for the a.s. and residues thereof in air**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March 2008
Materials and methods	Applicant's version is acceptable.
Conclusion	Applicant's version is adopted
Reliability	1
Acceptability	Acceptable
Remarks	/
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A4.2(b)_1 Mean recoveries for the determination of cypermethrin residues in air

Air condition	Mean recovery (%)			
	Analyte	LOQ	10x LOQ	Overall
Ambient (21.1 to 22.7°C / 50 to 56 % r.h)	total cypermethrin	84	103	93
	cis-I isomer	82	94	88
	trans-I isomer	84	98	91
	cis-II isomer	89	111	100
	trans-II isomer	80	110	95
Elevated (35.1 to 35.5°C / 82 to 99% r.h.)	total cypermethrin	82	91	87
	cis-I isomer	95	89	92
	trans-I isomer	70	85	77
	cis-II isomer	84	96	90
	trans-II isomer	89	99	94

Table A4.2(b)_2 %RSD for the determination of cypermethrin residues in air

Air condition	Precision (RSD%)			
	Analyte	LOQ	10x LOQ	Overall
Ambient (21.1 to 22.7°C / 50 to 56 % r.h)	total cypermethrin	9.7	12.5	15.3
	cis-I isomer	20.4	17.2	19.0
	trans-I isomer	9.9	9.9	12.3
	cis-II isomer	5.9	17.1	17.8
	trans-II isomer	8.6	12.0	19.6
Elevated (35.1 to 35.5°C / 82 to 99% r.h.)	total cypermethrin	12.1	5.4	10.2
	cis-I isomer	14.1	7.2	11.2
	trans-I isomer	16.2	4.7	14.5
	cis-II isomer	15.5	7.2	12.8
	trans-II isomer	11.0	3.9	9.5

Section A4 (4.2c) Analytical Methods for Detection and Identification

Annex Point IIA 4.2 (c) **Analytical method for the a.s. and residues thereof in water**

Official use only

		1 REFERENCE
1.1	**Reference**	Wimbush J (2002); Cypermethrin: Validation of an analytical method for the determination and confirmation of residues in surface water; Covance Laboratories Ltd, report no.40/040-D2149 (CYP/C69), 23 October 2002 (unpublished). Dates of experimental work: 7 May 2002 – 19 July 2002
1.2	**Data protection**	Yes
1.2.1	Data owner	Chimac-Agriphar s.a.
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes Requirements of Council Directive 91/414/EEC (15/07/91) as amended by Commission Directive 96/46/EC (16/07/96) and EU guidance document on residue analytical methods SANCO/825/00 rev. 6 (20/06/00).
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Preliminary treatment**	
3.1.1	Enrichment	Partition of water with hexane (three times), followed by evaporation of the combined organic phases, reconstitution of the dried extract in hexane.
3.1.2	Cleanup	Removal of co-extractives on a silica SPE cartridge. After evaporation the eluate is reconstituted in toluene.
3.2	**Detection**	
3.2.1	Separation method	Quantitative determination by capillary GC-ECD (HP-5, 30 m x 0.32 mm, 0.25 µm film thickness); confirmation by GC-MS (DB-5MS, 30 m x 0.25 mm, 0.25 µm film thickness)
3.2.2	Detector	Electron Capture Detection (quantitative) and Mass Spectrometric Detection (confirmatory) using chemical ionisation in the negative ion mode (ions monitored: m/z 207, 209 and 211). Each diastereoisomer of Cypermethrin is measured individually and the total cypermethrin residue is calculated by summing the 4 individual diastereoisomers.
3.2.3	Standard(s)	Calibration is achieved using an internal reference marker (permethrin) which is added to the final extract prior to analysis to compensate for any changes in detector response.
3.2.4	Interfering substance(s)	None
3.3	**Linearity**	
3.3.1	Calibration range	0.005 to 0.5 mg/L total cypermethrin

Section A4 (4.2c) **Analytical Methods for Detection and Identification**

Annex Point IIA 4.2 (c) **Analytical method for the a.s. and residues thereof in water**

3.3.2	Number of measurements	6 standard solutions of increasing cypermethrin concentration
3.3.3	Linearity	Correlation coefficient $r^2 > 0.99$
3.4	**Specifity: interfering substances**	No significant matrix interference (control values < 30% LOQ)
3.5	**Recovery rates at different levels**	See table A4.2(c)_1 and table A4.2(c)_2
3.5.1	Relative standard deviation	See table A4.2(c)_1 and table A4.2(c)_2
3.6	**Limit of determination**	Limit of Quantification (LOQ) = 0.01 µg/L (total cypermethrin)
3.7	**Precision**	
3.7.1	Repeatability	Not determined
3.7.2	Independent laboratory validation	Not determined

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	Gas Chromatography with Electron Capture Detection (GC-ECD), with confirmatory analysis by Gas Chromatography with Mass Spectrometric Detection (GC-MS with negative chemical ionisation). Each stereoisomer of cypermethrin is measured individually and the total cypermethrin residue calculated by summing the concentration of the four stereoisomers.
4.2	**Conclusion**	GC-ECD method (confirmation by GC-MSD) is suitable for the determination of residues of Cypermethrin in surface water, with an LOQ of 0.01 µg/L. The mean recoveries obtained for trans-II isomer at 0.01 µg/L level by both techniques were outside the acceptable range of 70-110%. However, as the purpose of the method is to quantify total cypermethrin and since the recoveries for total cypermethrin were acceptable at both fortification levels, this method is considered acceptable for measurement of cypermethrin residues in surface water.
4.2.1	Reliability	1
4.2.2	Deficiencies	No Study evaluated and accepted under Directive 91/414/EC.

Section A4 (4.2c) Analytical Methods for Detection and Identification

Annex Point IIA 4.2 (c) **Analytical method for the a.s. and residues thereof in water**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March 2008
Materials and methods	Applicants version is acceptable
Conclusion	Applicant's version is adopted
Reliability	*2*
Acceptability	Acceptable.
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A4.2(c)_1 Validation of method CLE 0040/040-04R for residues in water :

Primary method (GC-ECD)

Matrix	Analyte	Fortification level (µg/L commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
surface water	cis-I	0.0022	5	89 – 112	97	9.6
		0.0223	5	77 – 87	83	4.9
	trans-I	0.0035	5	88 – 111	96	9.3
		0.0346	5	76 – 85	82	4.5
	cis-II	0.0020	5	97 – 118	103	8.3
		0.0197	5	90 – 100	95	4.4
	trans-II	0.0023	5	103 – 127	**112**	8.5
		0.0234	5	97 – 109	103	4.5
	Cypermethrin (total isomers)	0.01	5	94 – 116	101	8.4
		0.10	5	84 – 94	89	4.6

Table A4.2(c)_2 Validation of method CLE 0040/040-04R for residues in water :

Confirmatory method (GC-MSD)

Matrix	Analyte	Fortification level (µg/L commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
surface water	cis-I	0.0022	5	76 – 102	84	12.5
		0.0223	5	72 – 88	81	7.7
	trans-I	0.0035	5	82 – 100	87	8.5
		0.0346	5	69 – 86	77	8.4
	cis-II	0.0020	5	85 – 100	94	7.1
		0.0197	5	88 – 107	97	8.0
	trans-II	0.0023	5	114 – 136	121	7.3
		0.0234	5	92 – 111	102	6.9
	Cypermethrin (total isomers)	0.01	5	89 – 108	93	7.6
		0.10	5	79 – 97	88	7.8

Section A4 (4.2) (d) Analytical Methods for Detection and Identification

Annex Point IIA 4.2 (d) **Analytical method for the a.s. and residues thereof in animal and human body tissues**

			Official use only
		1 REFERENCE	
1.1	**Reference**	Wimbush, J (2003); Cypermethrin: : Validation of an analytical method for the determination and confirmation of residues in products of animal origin (milk, liver, kidney, muscle, fat and eggs); Covance Laboratories Ltd, report no.40/041-D2149 (CYP/C68), 14 October 2002 – amended 23 April 2003 (unpublished) Dates of experimental work: 22 May 2002 – 22 July 2002 Devine H (2003); Independent laboratory validation of Covance method CLE 0040/041-01R for residues of cypermethrin in bovine muscle, fat and hen eggs; CEM Analytical Services Ltd, report no. CEMR-1934 (CYP/C75), 8 May 2003 (unpublished). Dates of experimental work: 4 March 2003 – 11 April 2003 Devine H (2003); Independent laboratory validation of Covance method CLE 0040/041-02RM for residues of cypermethrin in bovine milk; CEM Analytical Services Ltd, report no. CEMR-1935 (CYP/C74), 8 May 2003 (unpublished). Dates of experimental work: 6 March 2003 – 17 March 2003	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Chimac-Agriphar s.a.	
1.2.2			
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes Requirements of Council Directive 91/414/EEC (15/07/91) as amended by Commission Directive 96/46/EC (16/07/96) and EU guidance document on residue analytical methods SANCO/825/00 rev. 6 (20/06/00).	
2.2	**GLP**	Yes	
2.3	**Deviations**	Detection system produced only 2 ions in sufficient abundance for quantification, not three ions; but considered to be sufficiently self-confirmatory.	X
		3 MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	*Method CLE 0040/041-01R for bovine liver, kidney, muscle, fat and hen eggs :*Extraction of Cypermethrin residues with acetonitrile *Method CLE 0040/041-02R.M for bovine milk :*Extraction of Cypermethrin residues with potassium oxalate, ethanol, diethyl ether and hexane, after which the diethyl ether/hexane layer is separated and transferred, and the remaining ethanol/milk mixture is further extracted with hexane.	
3.1.2	Cleanup	*Method CLE 0040/041-01R:* Clean-up with hexane partition,	

Section A4 (4.2) (d)	Analytical Methods for Detection and Identification		
Annex Point IIA 4.2 (d)	Analytical method for the a.s. and residues thereof in animal and human body tissues		
		evaporation to dryness and reconstitution in hexane, and removal of co-extractives by silica SPE cartridge. The SPE eluate is evaporated to dryness and reconstituted in internal standard solution (permethrin in toluene) *Method CLE 0040/041-02R.M:* The hexane and diethyl ether/hexane extracts are combined, evaporated to dryness, reconstituted with hexane and partitioned with acetonitrile. The acetonitrile layer is evaporated to dryness and reconstituted in internal standard solution (permethrin in toluene).	
3.2	**Detection**		
3.2.1	Separation method	Quantitative determination by capillary GC (DB-5MS, 30 m x 0.25 mm, 0.25 µm film thickness)	
3.2.2	Detector	Mass Spectrometric Detection (MSD) using chemical ionisation in the negative ion mode (ions monitored: m/z 207, 209 and 211; ions for quantification : m/z 207 and 209) . Each diastereoisomer of Cypermethrin is measured individually and the total cypermethrin residue is calculated by summing the 4 individual diastereoisomers.	
3.2.3	Standard(s)	Internal standard solution (permethrin in toluene)	
3.2.4	Interfering substance(s)	None	
3.3	**Linearity**		
3.3.1	Calibration range	0.01 to 1 mg/L total cypermethrin	
3.3.2	Number of measurements	6 standard solutions of increasing cypermethrin concentration	
3.3.3	Linearity	Correlation coefficient $r^2 > 0.98$	
3.4	**Specifity: interfering substances**	No significant matrix interference (control values < 30% LOQ)	
3.5	**Recovery rates at different levels**	See table A4.2(d)_1 and table A4.2(d)_2	
3.5.1	Relative standard deviation	See table A4.2(d)_1 and table A4.2(d)_2	
3.6	**Limit of determination**	Limit of Quantification (LOQ) (total cypermethrin): 0.05 mg/kg (bovine edible tissue : muscle, liver, kidney, fat) 0.005 mg/kg (bovine milk) 0.01 mg/kg (hen eggs)	
3.7	**Precision**		
3.7.1	Repeatability	Not determined	X
3.7.2	Independent laboratory validation	First validation by Covance Laboratories; ILV by CEMAS.	

Section A4 (4.2) (d) Analytical Methods for Detection and Identification

Annex Point IIA 4.2 (d) **Analytical method for the a.s. and residues thereof in animal and human body tissues**

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1 Materials and methods

Method CLE 0040/041-01R for bovine liver, kidney, muscle, fat and hen eggs :

Extraction of Cypermethrin residues with acetonitrile followed by reconstitution in hexane and removal of co-extractives by silica SPE cartridge. Quantitative determination by capillary GC/MSD using chemical ionisation in the negative ion mode (ions monitored: m/z 207, 209 and 211; ions for quantification : m/z 207 and 209) . Each diastereoisomer of Cypermethrin is measured individually and the total cypermethrin residue is calculated by summing the 4 individual diastereoisomers.

Method CLE 0040/041-02R.M for bovine milk :

Extraction of Cypermethrin residues with potassium oxalate, ethanol, diethyl ether and hexane, after which the diethyl ether/hexane layer is separated and transferred, and the remaining ethanol/milk mixture is further extracted with hexane. The hexane and dietyl ether/hexane extracts are combined, evaporated to dryness, reconstituted with hexane and partitioned with acetonitrile. The acetonitrile layer is evaporated to dryness and reconstituted in internal standard solution. Quantitative determination as indicated above.

4.2 Conclusion

GC-MSD methods are suitable for the determination of residues of Cypermethrin in products of animal origin, with an LOQ of 0.05 mg/kg for bovine edible tissues (muscle, liver, kidney, fat), 0.005 mg/kg for bovine milk and 0.01 mg/kg for hen eggs. Method has been successfully validated by an independent laboratory X

4.2.1 Reliability 1

4.2.2 Deficiencies No

Studies evaluated and accepted under Directive 91/414/EC.

Section A4 (4.2) (d) Analytical Methods for Detection and Identification

Annex Point IIA 4.2 (d) **Analytical method for the a.s. and residues thereof in animal and human body tissues**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March, 2008.
Materials and methods	The applicant's version is acceptable with the following amendments: 2.3. deviations: Detection system produced only 2 ions in sufficient abundance for quantification, not three ions; but considered to be sufficiently self-confirmatory. 3.7.1 repeatability: Analysis of each validation level in quintuplicate. Precision of the method was acceptable (RSD ≤ 20%) for each stereoisomer and total cypermethrin.
Conclusion	The applicant's version is adopted with the following amendments: GC-MSD methods, with chemical ionisation in the negative ion mode, are suitable for the determination of residues of Cypermethrin in products of animal origin, with an LOQ of 0.05 mg/kg for bovine edible tissues (muscle, liver, kidney, fat), 0.005 mg/kg for bovine milk and 0.01 mg/kg for hen eggs. Specificity: No interference > 30% of LOQ in the control matrices. Repeatability: RSD values < 20% mean recoveries between 70% and 110% Method has been successfully validated by an independent laboratory
Reliability	1
Acceptability	Acceptable.
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A4.2(d)_1 Validation of methods for residues in products of animal origin (ion quantified : m/z 207)

Matrix	Analyte	Fortification level (mg/kg commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
First validation (Wimbush, 2003)						
bovine muscle	cis-I	0.0112	5	84 – 90	86	3.0
		0.1115	5	79 – 84	81	2.2
	trans-I	0.0173	5	83 – 88	85	2.5
		0.1730	5	79 – 85	81	2.8
	cis-II	0.0099	5	85 – 93	89	3.6
		0.0985	5	79 – 84	81	2.2
	trans-II	0.0117	5	89 – 98	92	4.0
		0.1170	5	79 – 85	81	2.8
	Cypermethrin (total isomers)	0.05	5	86 – 91	87	2.5
		0.50	5	80 – 84	81	2.2
bovine kidney	cis-I	0.0112	5	93 – 101	97	2.9
		0.1115	5	85 – 88	86	1.8
	trans-I	0.0173	5	91 – 101	96	3.9
		0.1730	5	86 – 92	88	2.8
	cis-II	0.0099	5	98 – 108	104	3.9
		0.0985	5	83 – 89	86	2.6
	trans-II	0.0117	5	98 – 108	103	3.8
		0.1170	5	80 – 87	84	3.1
	Cypermethrin	0.05	5	95 – 103	100	3.0
	(total isomers)	0.50	5	84 – 89	87	2.3
bovine liver	cis-I	0.0112	5	77 – 99	87	10.4
		0.1115	5	83 – 90	86	3.2
	trans-I	0.0173	5	73 – 89	83	7.7
		0.1730	5	83 – 91	87	3.9
	cis-II	0.0099	5	76 – 95	87	8.0
		0.0985	5	83 – 92	87	3.9
	trans-II	0.0117	5	68 – 100	82	14.2
		0.1170	5	73 – 90	84	8.2
	Cypermethrin	0.05	5	83 – 87	85	2.1
	(total isomers)	0.50	5	81 – 90	86	4.5
bovine fat	cis-I	0.0112	5	77 – 89	84	6.5
		0.1115	5	93 – 102	98	3.9

Matrix	Analyte	Fortification level (mg/kg commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
(bovine fat cont.)	trans-I	0.0173	5	76 – 81	78	3.2
		0.1730	5	94 – 104	99	4.0
	cis-II	0.0099	5	78 – 83	81	2.7
		0.0985	5	90 – 99	94	4.4
	trans-II	0.0117	5	80 – 90	84	4.8
		0.1170	5	90 – 96	94	3.0
	Cypermethrin	0.05	5	78 – 84	82	3.5
	(total isomers)	0.50	5	93 – 101	97	37
hen eggs	cis-I*	0.0022	5	80 – 90	85	5.2
		0.0223	5	88 – 95	91	3.9
	trans-I*	0.0035	5	76 – 89	81	6.7
		0.0346	5	84 – 90	87	3.0
	cis-II*	0.0020	5	80 – 89	85	4.4
		0.0197	5	86 – 94	91	3.3
	trans-II*	0.0023	5	81 – 87	84	3.2
		0.0234	5	91 – 100	96	3.3
	Cypermethrin*	0.01	5	80 – 87	83	3.9
	(total isomers)	0.10	5	87 – 94	91	3.1
bovine milk	cis-I*	0.0011	5	102 – 118	110	5.4
		0.0112	5	70 – 99	87	13.9
	trans-I*	0.0017	5	80 – 99	87	8.5
		0.0173	5	59 – 85	74	14.9
	cis-II*	0.0010	5	68 – 102	84	15.7
		0.0099	5	59 – 87	73	15.3
	trans-II*	0.0012	5	79 – 107	90	12.6
		0.0117	5	62 – 93	77	16.1
	Cypermethrin*	0.005	5	84 – 106	92	9.7
	(total isomers)	0.050	5	62 – 90	77	15.1
Independent lab validation (Devine, 2003)						
bovine muscle	cis-I	0.0120	5	81 – 83	81	1.1
		0.1195	5	77 – 89	84	5.5
	trans-I	0.0165	5	83 – 86	85	1.3
		0.1650	5	82 – 96	90	6.3
	cis-II	0.0102	5	75 – 83	79	3.6
		0.1020	5	73 – 81	78	4.1

Matrix	Analyte	Fortification level (mg/kg commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
(bovine muscle cont.)	trans-II	0.0114 0.1135	5 5	84 – 88 76 – 88	86 83	2.1 5.3
	Cypermethrin (total isomers)	0.05 0.5	5 5	82 – 85 78 – 89	83 85	1.3 5.2
bovine fat	cis-I	0.0120 0.1195	5 5	86 – 97 73 – 88	93 80	4.8 6.7
	trans-I	0.0165 0.1650	5 5	91 – 100 72 – 86	96 79	3.4 6.4
	cis-II	0.0102 0.1020	5 5	91 – 110 71 – 85	99 78	7.3 6.6
	trans-II	0.0114 0.1135	5 5	94 – 108 71 – 86	99 79	5.6 7.2
	Cypermethrin (total isomers)	0.05 0.5	5 5	92 – 101 72 – 86	96 79	4.1 6.5
hen eggs	cis-I	0.0024 0.0239	5 5	91 – 101 83 – 86	96 84	3.8 1.5
	trans-I	0.0033 0.0330	5 5	101 – 108 83 – 86	105 84	2.5 1.5
	cis-II	0.0020 0.0204	5 5	96 – 107 83 – 86	103 84	4.7 1.5
	trans-II	0.0023 0.0227	5 5	96 – 101 84 – 89	98 86	2.0 2.1
	Cypermethrin (total isomers)	0.01 0.1	5 5	98 – 102 84 – 86	101 85	1.9 1.1
Independent lab validation (Devine, 2003)						
bovine milk	cis-I	0.0012 0.0120	5 5	80 – 91 89 – 96	87 92	4.8 3.6
	trans-I	0.0017 0.0165	5 5	72 – 87 89 – 99	81 94	7.1 5.0
	cis-II	0.0010 0.0102	5 5	68 – 82 89 – 100	76 95	7.2 4.7
	trans-II	0.0011 0.0114	5 5	70 – 92 98 – 110	82 105	9.8 4.8
	Cypermethrin (total isomers)	0.005 0.05	5 5	73 – 88 91 – 101	82 96	6.8 4.3

* recovery corrected for control value (< 30% of LOQ)

Table A4.2(d)_2 Validation of methods for residues in products of animal origin (ion quantified : m/z 209)

Matrix	Analyte	Fortification level (mg/kg commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
bovine muscle	cis-I	0.0112	5	85 – 93	88	3.8
		0.1115	5	79 – 83	81	2.0
	trans-I	0.0173	5	83 – 88	86	2.2
		0.1730	5	80 – 86	82	3.2
	cis-II	0.0099	5	84 – 94	88	4.6
		0.0985	5	78 – 84	80	2.9
	trans-II	0.0117	5	92 – 94	93	1.2
		0.1170	5	78 – 84	80	3.1
	Cypermethrin (total isomers)	0.05	5	87 – 92	89	2.6
		0.50	5	79 – 84	81	2.5
bovine kidney	cis-I	0.0112	5	95 – 106	101	4.1
		0.1115	5	84 – 88	86	1.8
	trans-I	0.0173	5	96 – 105	99	3.5
		0.1730	5	87 – 92	89	2.6
	cis-II	0.0099	5	98 – 108	105	4.0
		0.0985	5	83 – 88	85	2.7
	trans-II	0.0117	5	99 – 111	106	5.0
		0.1170	5	81 – 86	84	2.6
	Cypermethrin (total isomers)	0.05	5	97 – 106	103	3.3
		0.50	5	85 – 89	87	2.1
bovine liver	cis-I	0.0112	5	84 – 112	95	11.3
		0.1115	5	87 – 90	88	1.2
	trans-I	0.0173	5	71 – 98	86	11.5
		0.1730	5	85 – 90	88	2.5
	cis-II	0.0099	5	72 – 105	95	13.9
		0.0985	5	88 – 90	89	1.0
	trans-II	0.0117	5	83 – 115	97	13.7
		0.1170	5	90 – 94	92	2.0
	Cypermethrin (total isomers)	0.05	5	83 – 104	92	9.2
		0.50	5	87 – 91	89	1.8
bovine fat	cis-I	0.0112	5	79 – 89	84	4.9
		0.1115	5	92 – 101	97	4.2

Matrix	Analyte	Fortification level (mg/kg commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
(Bovine fat cont.)	trans-I	0.0173	5	77 – 83	80	3.0
		0.1730	5	92 – 102	97	4.7
	cis-II	0.0099	5	82 – 86	84	1.8
		0.0985	5	87 – 97	93	4.7
	trans-II	0.0117	5	81 – 98	87	7.4
		0.1170	5	89 – 96	93	3.6
	Cypermethrin	0.05	5	80 – 88	83	3.7
	(total isomers)	0.50	5	91 – 99	95	4.3
hen eggs	cis-I*	0.0022	5	80 – 95	85	7.3
		0.0223	5	86 – 97	91	5.6
	trans-I*	0.0035	5	77 – 81	78	2.5
		0.0346	5	83 – 93	87	5.0
	cis-II*	0.0020	5	80 – 88	84	3.6
		0.0197	5	86 – 97	91	4.4
	trans-II*	0.0023	5	80 – 87	84	3.2
		0.0234	5	89 – 102	95	5.2
	Cypermethrin*	0.01	5	80 – 84	82	2.5
	(total isomers)	0.10	5	85 – 97	91	5.2
bovine milk	cis-I*	0.0011	5	94 – 118	104	9.1
		0.0112	5	70 – 97	86	13.2
	trans-I*	0.0017	5	79 – 100	86	10.3
		0.0173	5	59 – 83	73	14.3
	cis-II*	0.0010	5	70 – 96	82	13.7
		0.0099	5	59 – 85	73	14.7
	trans-II*	0.0012	5	76 – 106	89	13.7
		0.0117	5	60 – 91	76	16.4
	Cypermethrin*	0.005	5	82 – 105	90	10.8
	(total isomers)	0.050	5	62 – 88	76	14.5

* recovery corrected for control value (< 30% of LOQ)

Section A4 (4.3) Analytical Methods for Detection and Identification

Annex Point IIA 4.3 **Analytical method for the a.s. and residues thereof in/on food or feedstuffs**

Official use only

1 REFERENCE

1.1	**Reference**	Wimbush, J (2003); Cypermethrin: Validation of the DFG multi residue method S23 for the determination and confirmation of residues in oilseed rape (seed, oil and straw) and wheat (grain and straw); Covance Laboratories Ltd, report no.40/037-D2149 (CYP/C67), 28 August 2002 (unpublished). Dates of experimental work: 6 March 2002 – 19 April 2002 Devine H (2003); Independent laboratory validation of Covance method CLE 0040/037-03RO for residues of cypermethrin in oilseed rape (oil); CEM Analytical Services Ltd, report no. CEMR-1933 (CYP/C73), 7 May 2003 (unpublished). Dates of experimental work: 4 February 2003 – 20 February 2003 Devine H (2003); Independent laboratory validation of Covance method CLE 0040/037-03R for residues of cypermethrin in oilseed rape (seed) and wheat grain; CEM Analytical Services Ltd, report no. CEMR-1932 (CYP/C72), 7 May 2003 (unpublished). Dates of experimental work: 7 February 2003 – 1 March 2003
1.2	**Data protection**	Yes
1.2.1	Data owner	Chimac-Agriphar s.a.
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes Requirements of Council Directive 91/414/EEC (15/07/91) as amended by Commission Directive 96/46/EC (16/07/96) and EU guidance document on residue analytical methods SANCO/825/00 rev. 6 (20/06/00).
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Preliminary treatment**	*Method CLE 0040/037-03R for oilseed rape (seed) and wheat (grain and straw) :* Extraction of Cypermethrin residues with hexane/acetone (80:20 v/v), followed by acetonitrile – hexane partition (oilseed rape only) and removal of co-extractives by Florisil column chromatography. Quantitative determination by capillary GC-ECD (HP-5, 30 m x 0.32 mm, 0.25 µm film thickness). Confirmatory analysis used the same technique with a capillary column of different polarity (DB-17MS, 15 m x 0.25 mm, 0.25 µm film thickness). Each diastereoisomer of Cypermethrin is measured individually and the total cypermethrin residue is calculated by summing the 4 individual diastereoisomers. Quantification by external standardization (cypermethrin standard; percentage of each diastereoisomer certified).

Section A4 (4.3)		Analytical Methods for Detection and Identification
Annex Point IIA 4.3		**Analytical method for the a.s. and residues thereof in/on food or feedstuffs**
		Method CLE 0040/037-03RO for oilseed rape (oil) : Extraction of Cypermethrin residues with hexane, followed by acetonitrile-hexane partition and removal of co-extractives by Florisil column chromatography. Quantitative determination as indicated above.
3.1.1	Enrichment	*Method CLE 0040/037-03R for oilseed rape (seed) and wheat (grain and straw) :* Extraction of Cypermethrin residues with hexane/acetone (80:20 v/v), followed by acetonitrile – hexane partition (oilseed rape only) and removal of co-extractives by Florisil column chromatography. *Method CLE 0040/037-03RO for oilseed rape (oil) :* Extraction of Cypermethrin residues with hexane, followed by acetonitrile-hexane partition and removal of co-extractives by Florisil column chromatography.
3.1.2	Cleanup	Florisil column chromatography
3.2	**Detection**	
3.2.1	Separation method	Quantitative determination by capillary GC-ECD (HP-5, 30 m x 0.32 mm, 0.25 µm film thickness). Confirmatory analysis used the same technique with a capillary column of different polarity (DB-17MS, 15 m x 0.25 mm, 0.25 µm film thickness). Each diastereoisomer of Cypermethrin is measured individually and the total cypermethrin residue is calculated by summing the 4 individual diastereoisomers.
3.2.2	Detector	Electron capture (ECD)
3.2.3	Standard(s)	Quantification by external standardization (cypermethrin standard; percentage of each diastereoisomer certified).
3.2.4	Interfering substance(s)	None
3.3	**Linearity**	
3.3.1	Calibration range	0.02 to 1.5 mg/L total cypermethrin
3.3.2	Number of measurements	5
3.3.3	Linearity	Response of GC-ECD system (HP5) to each diastereoisomer (peak area vs. conc) was demonstrated to be linear within a concentration range of 0.02 to 1.5 mg/L total cypermethrin ($n>6$), $r^2 > 0.98$.

Section A4 (4.3)	**Analytical Methods for Detection and Identification**
Annex Point IIA 4.3	**Analytical method for the a.s. and residues thereof in/on food or feedstuffs**

3.4	**Specifity: interfering substances**	Control extracts of each matrix were shown to be free from components that interfered with cypermethrin, with the exception of oilseed rape seed, which contained a component that interfered with the analysis of the cis-I isomer and oilseed rape oil, which contained a component that interfered with the analysis of each diastereoisomer. However the mean concentrations of these interfering components in the control samples did not exceed 30% of the LOQ, so the methods were considered to be specific for cypermethrin.
3.5	**Recovery rates at different levels**	Recovery was determined over the concentration ranges 0.05 - 0.5 mg/kg for cypermethrin residues in oilseed rape (seed and oil) and 0.025 - 0.25 mg/kg for cypermethrin residues in wheat (grain and straw). The mean recovery of each of the isomers was within the acceptance criteria of 70% to 110%, as defined in the Uniform Principles decision-making criteria, with the exception of the cis-I, trans-I and trans-II isomers in wheat straw at 0.025 mg/kg which had recoveries of 125%, 114% and 115% respectively. Despite these high recoveries for the individual isomers, the overall recovery for total cypermethrin in wheat straw was 110% at 0.025 mg/kg which is within the acceptable range. See tables A4.3_1 and A4.3_2
3.6	**Limit of determination**	0.05 mg/kg for oilseed rape (seed and oil) 0.025 mg/kg for wheat (grain and straw)
3.7	**Precision**	
3.7.1	Repeatability	Repeatability of the methods, expressed as the relative standard deviation (RSD) of the recovery measurements ranged from 1.8% to 18.1% for each individual isomer and for total cypermethrin. RSD values at each validation level for each matrix were less than 20% which is also indicative of good repeatability.
3.7.2	Independent laboratory validation	First validation by Covance Laboratories; ILV by CEMAS. See table A4.3_1

Section A4 (4.3)	**Analytical Methods for Detection and Identification**
Annex Point IIA 4.3	**Analytical method for the a.s. and residues thereof in/on food or feedstuffs**

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	Method based on DFG S23 multi residue method for pyrethroids Extraction of Cypermethrin residues with hexane/acetone. Quantitative determination by capillary GC-ECD (HP-5, 30 m x 0.32 mm, 0.25 µm film thickness). Confirmatory analysis used the same technique with a capillary column of different polarity. Total cypermethrin residue is calculated by summing the 4 individual diastereoisomers. Quantification by external standardization.
4.2	**Conclusion**	GC-ECD method is suitable for the determination of residues of Cypermethrin in wheat and oilseed rape, with an LOQ of 0.05 mg/kg for oilseed rape and 0.025 mg/kg for wheat. Independent lab validation was addressed in an acceptable manner.
4.2.1	Reliability	1
4.2.2	Deficiencies	No Studies evaluated and accepted under Directive 91/414/EC.

Section A4 (4.3) Analytical Methods for Detection and Identification

Annex Point IIA 4.3 **Analytical method for the a.s. and residues thereof in/on food or feedstuffs**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March, 2008.
Materials and methods	The applicant's version is acceptable.
Conclusion	The applicant's version is adopted: The adapted DFG multi-residue method S23 (GC-ECD method) is suitable for the determination of residues of Cypermethrin in wheat and oilseed rape, with an LOQ of 0.05 mg/kg for oilseed rape and 0.025 mg/kg for wheat. Specificity: No interference > 30% of LOQ in the control matrices. Mean recoveries between 70% and 110% Repeatability: RSD values < 20% Independent lab validation was addressed in an acceptable manner.
Reliability	1
Acceptability	Acceptable
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A4.3_1: Validation of methods for residues in crops : primary method (GC-ECD on non-polar column)

Matrix	Analyte	Fortification level (mg/kg commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
First validation (Wimbush, 2002)						
oilseed rape (seed)	cis-I	0.0112	5	80 – 94	85	6.8
		0.1115	5	83 – 92	87	4.4
	trans-I	0.0173	5	78 – 91	86	6.0
		0.1730	5	82 – 92	87	5.4
	cis-II	0.0099	5	92 – 121	104	11.8
		0.0985	5	77 – 90	83	6.4
	trans-II	0.0117	5	71 – 89	82	8.3
		0.1170	5	76 – 90	83	6.9
	Cypermethrin (total isomers)	0.05	5	80 – 94	89	6.6
		0.50	5	80 – 91	85	5.7
oilseed rape (oil)	cis-I*	0.0112	5	75 – 87	82	5.5
		0.1115	5	76 - 84	79	4.0
	trans-I*	0.0173	5	92 – 104	97	4.9
		0.1730	5	79 – 88	83	4.9
	cis-II*	0.0099	5	83 – 91	88	3.7
		0.0985	5	72 – 80	76	4.7
	trans-II*	0.0117	5	81 – 90	86	3.8
		0.1170	5	73 – 80	77	4.0
	Cypermethrin* (total isomers)	0.05	5	87 – 94	89	3.0
		0.50	5	76 – 82	79	3.4
wheat (grain)	cis-I	0.0056	5	83 – 96	89	5.8
		0.0558	5	92 – 96	94	1.8
	trans-I	0.0087	5	81 – 96	89	6.4
		0.0865	5	90 – 95	93	2.1
	cis-II	0.0049	5	52 – 88	75	18.1
		0.0493	5	60 – 85	77	12.9
	trans-II	0.0059	5	62 – 91	79	13.3
		0.0585	5	64 – 88	80	11.7
	Cypermethrin (total isomers)	0.025	5	71 – 93	84	9.7
		0.25	5	79 – 92	87	5.6

wheat (straw)	cis-I	0.0056 0.0558	5 5	117 – 136 89 – 99	**125** 95	5.5 4.2
	trans-I	0.0087 0.0865	5 5	106 – 119 87 – 98	**114** 93	4.4 4.5
	cis-II	0.0049 0.0493	5 5	76 – 89 75 – 86	81 80	6.4 5.4
	trans-II	0.0059 0.0585	5 5	111 – 125 82 – 94	**115** 88	4.8 5.6
	Cypermethrin (total isomers)	0.025 0.25	5 5	104 – 117 84 – 95	110 90	4.3 4.8
Independent lab validation (Devine, 2003)						
oilseed rape (seed)	cis-I	0.0120 0.1195	5 5	82 – 89 79 – 93	84 89	3.3 6.2
	trans-I	0.0165 0.1650	5 5	72 – 84 79 – 89	78 86	5.7 4.7
	cis-II	0.0102 0.1020	5 5	75 – 87 77 – 87	83 84	6.2 4.9
	trans-II	0.0114 0.1135	5 5	71 – 80 75 – 87	75 83	5.1 5.9
	Cypermethrin (total isomers)	0.05 0.50	5 5	75 – 85 78 – 88	79 85	4.8 5.1
wheat (grain)	cis-I	0.0060 0.0598	5 5	76 – 88 70 – 81	84 76	5.6 7.1
	trans-I	0.0083 0.0825	5 5	68 – 77 65 – 82	75 74	5.2 9.4
	cis-II	0.0051 0.0510	5 5	67 – 79 59 – 79	75 **69**	6.6 11.0
	trans-II	0.0057 0.0568	5 5	66 – 78 58 - 77	73 **68**	7.4 10.8
	Cypermethrin (total isomers)	0.025 0.25	5 5	69 – 80 64 – 80	77 72	6.0 9.1

Independent lab validation (Devine, 2003)						
oilseed rape (oil)	cis-I	0.0120	5	93 – 116	105	9.4
		0.1195	5	74 – 88	81	7.8
	trans-I	0.0165	5	96 – 120	107	9.9
		0.1650	5	69 – 89	79	9.9
	cis-II	0.0102	5	55 – 89	72	19.7
		0.1020	5	67 – 86	75	10.2
	trans-II	0.0114	5	95 – 124	110	10.8
		0.1135	5	66 – 88	77	11.2
	Cypermethrin (total isomers)	0.05	5	87 – 113	100	11.4
		0.50	5	69 – 88	78	9.5

* recovery corrected for control value (< 30% of LOQ)

Table A4.3_2: Validation of method for residues in crops : confirmatory method (GC-ECD on intermediately polar column)

Matrix	Analyte	Fortification level (mg/kg commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
oilseed rape (seed)	cis-I*	0.0112	5	81 – 97	88	7.1
		0.1115	5	88 – 101	94	6.2
	trans-I	0.0173	5	99 – 113	104	5.7
		0.1730	5	88 – 98	93	4.9
	cis-II	0.0099	5	91 – 107	99	6.9
		0.0985	5	86 – 96	91	4.3
	trans-II	0.0117	5	92 – 104	98	5.9
		0.1170	5	85 – 96	90	5.5
	Cypermethrin* (total isomers)	0.05	5	93 – 106	98	5.8
		0.50	5	88 – 97	92	5.2
oilseed rape (oil)	cis-I*	0.0112	5	67 – 82	76	7.7
		0.1115	5	76 – 82	78	3.4
	trans-I*	0.0173	5	73 – 86	79	6.8
		0.1730	5	78 – 82	80	2.3
	cis-II*	0.0099	5	63 – 73	69	5.4
		0.0985	5	67 – 81	74	7.3
	trans-II*	0.0117	5	71 – 78	75	3.9
		0.1170	5	73 – 84	79	5.4
	Cypermethrin* (total isomers)	0.05	5	73 – 80	75	3.8
		0.50	5	76 – 82	78	3.4

Matrix	Analyte	Fortification level (mg/kg commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
wheat (grain)	cis-I	0.0056	5	106 – 111	108	1.8
		0.0558	5	105 – 106	106	0.5
	trans-I	0.0087	5	98 – 106	103	2.9
		0.0865	5	100 – 109	104	3.1
	cis-II	0.0049	5	100 – 104	101	1.7
		0.0493	5	66 – 99	89	14.6
	trans-II	0.0059	5	104 – 112	107	3.1
		0.0585	5	70 – 98	90	12.7
	Cypermethrin (total isomers)	0.025	5	101 – 106	105	2.0
		0.25	5	87 – 102	98	6.5
wheat (straw)	cis-I	0.0056	5	80 – 88	84	3.6
		0.0558	5	92 – 106	97	5.9
	trans-I	0.0087	5	104 – 111	108	3.0
		0.0865	5	97 – 108	100	4.8
	cis-II	0.0049	5	87 – 96	92	3.7
		0.0493	5	88 – 103	95	7.2
	trans-II	0.0059	5	79 – 89	84	5.4
		0.0585	5	90 – 104	96	6.5
	Cypermethrin (total isomers)	0.025	5	90 – 98	94	3.4
		0.25	5	93 – 105	97	5.3

Section A5 Effectiveness against target organisms and intended uses

Subsection (Annex Point)			Official use only
5.1	**Function (IIA5.1)**	Insecticide	
5.2	**Organism(s) to be controlled and products, organisms or objects to be protected (IIA5.2)**	For use as a Wood Preservative (Product Type 8.01 and 8.02).	
5.2.1	**Organism(s) to be controlled (IIA5.2)**	Wood destroying insects including; *Hylotrupes bajulus* (furniture beetle) *Anobium punctatum* (woodworm) *Reticulitermes santonesis* (termites)	
5.2.2	**Products, organisms or objects to be protected (IIA5.2)**	All Types of wood in use (hazard) classes 1, 2 and 3	
5.3	**Effects on target organisms, and likely concentration at which the active substance will be used (IIA5.3)**		
5.3.1	**Effects on target organisms (IIA5.3)**	Cypermethrin is a broad spectrum insecticide with contact and stomach action. Existing data from the public domain on the effectiveness of the active ingredient cypermethrin is presented in DocIIA-5.2/01-04.	
5.3.2	**Likely concentrations at which the A.S. will be used (IIA5.3)**	The concentration of the a.s. used depends on the nature of the type of treatment and the target species, with treatment of termites in Southern Europe usually requiring a higher concentration: Superficial treatment (dipping): 0.1 – 0.2 % cypermethrin Impregnation (vacuum-pressure): 0.05 % cypermethrin Industrial spray applications (preventative): 0.3 % cypermethrin Professional spray (preventative and remedial): 0.1% cypermethrin Amateur spray (preventative and remedial): 0.1% cypermethrin Professional brushing (preventative and remedial): 0.1% cypermethrin Amateur brushing (preventative and remedial): 0.1% cypermethrin	

Section A5		Effectiveness against target organisms and intended uses
5.4	**Mode of action (including time delay) (IIA5.4)**	
5.4.1	**Mode of action**	Cypermethrin is a synthetic pyrethroid with contact and stomach action. It acts by preventing the transmission of impulses along the nervous system of the insect. It is thought that this is achieved by blocking the sodium channels in nerve membranes, thus preventing action potentials passing down the nerve axon.
5.4.2	**Time delay**	As the action of cypermethrin does not depend on conversion or degradation into active form, this results in rapid mortality following contact.
5.5	**Field of use envisaged (IIA5.5)**	Product Type PT 8.01 and 8.02 – Wood Preservative, indoor and outdoor use (hazard classes 1-3)
5.6	**User (IIA5.6)**	
Industrial (PT08)		Industrial pre-treatment (dipping, vacuum-pressure, spraying cabinets).
Professional (PT08)		Professional spray and brush application (preventative and remedial treatment)
General public (PT08)		Amateur spray and brush application (preventative and remedial treatment)
5.7	**Information on the occurrence or possible occurrence of the development of resistance and appropriate management strategies (IIA5.7)**	
5.7.1	**Development of resistance**	Resistance to pyrethroid insecticides has been reported for a number of pests both in agriculture and public health.
5.7.2	**Management strategies**	Strategies such as alteration of insecticides with different modes of action and avoidance of over frequent use are standard practises in agriculture and should be applied also to biocidal uses of cypermethrin. In comparison, the biocidal market for cypermethrin is much smaller and products typically use a very low concentration of the active substance, hence the development of resistance should be lower.
5.8	**Likely tonnage to be placed on the market per year (IIA5.8)**	It is estimated that the market potential for cypermethrin in PT08 is approximately XXX tonnes / year. Other biocidal uses (PT18) are estimated to be XXX tonnes / year. By comparison, the estimated tonnage of cypermethrin sold for plant protection is in the region of XXX tonnes / year. See confidential Annex doc III A5.0_PT8_conf for more information

Section A5 Effectiveness against target organisms and intended uses

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	**25/11/2007**
Materials and methods	**The applicant's version is considered acceptable**
Conclusion	**Adopt applicant´s version**
Reliability	**The information given above is considered to be reliable. Many studies have been conducted to show effectiveness of cypermethrin against target organisms; the common reliability indicator that can be given is that all test methods presented are based on relevant EN standards.**
Acceptability	**acceptable**
Remarks	**none**
	COMMENTS FROM ...
Date	Give date of comments submitted
Results and discussion	Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant´s summary and conclusion. Discuss if deviating from view of rapporteur member state
Conclusion	Discuss if deviating from view of rapporteur member state
Reliability	Discuss if deviating from view of rapporteur member state
Acceptability	Discuss if deviating from view of rapporteur member state
Remarks	

Section 5.3: Summary table of experimental data on the effectiveness of the active substance against target organisms at different fields of use envisaged, where applicable

Function	**Field of use envisaged**	**Test substance**	**Test organism(s)**	**Test method**	**Test conditions**	**Test results: effects, mode of action, resistance**	**Reference*)**
Insecticide	08	Cypermethrin	House longhorn beetle (*Hylotrupes bajulus*) – cat.1	EN 47	Impregnation using 0.22-5.70 g/m^3	Toxic value 1.11 – 5.70 g/m^3	Anon (1981), BRE 80/11 (CYP/E28)
Insecticide	08	Cypermethrin	House longhorn beetle (*Hylotrupes bajulus*) – cat.2	EN 47	Impregnation using 3.30 – 13.35 g/m^3	Toxic value13.35 – 6.70 g/m^3	Anon (1981), BRE 80/12-15(CYP/E28)
Insecticide	08	Cypermethrin	Furniture beetle (*Anobium punctatum*)	EN 21	Impregnation using 9.7 – 39.3 g/m^3	Toxic value 20.3 – 39.3 g/m^3	Anon (1981), BRE (CYP/E28)
Insecticide	08	Cypermethrin (organic solvent and emulsion)	Furniture beetle (*Anobium punctatum*)	EN 21, EN 49, EN 48	Impregnation using 0.35 – 39.3 g/m^3	Toxic value 0.67 – 1.54 g/m^3 (egg laying) Toxic value 20.3 – 39.3 g/m^3 (mature larvae)	Read, S.J., Berry, R.W. (1984)
			House longhorn beetle (*Hylotrupes bajulus*)	EN 46, EN 47, EN 22	Impregnation using 0.2 – 13.4 g/m^3	Toxic value 1.1 – 5.7 g/m^3(egg laying) Toxic value 6.7 – 13.4 g/m^3 (mature larvae)	
			Termites (*Reticulitermes santonensis)*	EN 117, EN 118	Impregnation using 24.6 – 414.4 g/m^3	Toxic value 49.7 – 100 g/m^3	

References:

Study Summary / Annex point	Author	Year	Reference
IIIA5.2/01	Anon.	1981	Determination of toxic values against *Hylotrupes bajulus* larvae. Building Research Establishment, Princes Risborough Laboratory, UK; report no. 80/11 (CYP/E28).
IIIA5.2/02	Anon.	1981	Determination of toxic values against *Hylotrupes bajulus* larvae. Building Research Establishment, Princes Risborough Laboratory, UK; report no. 80/12-15 (CYP/E28).
IIIA5.2/03	Anon.	1980	Determination of toxic values against *Anobium punctatum* larvae. Building Research Establishment, Princes Risborough Laboratory, UK; (CYP/E28).
IIIA5.2/04	Read S.J., Berry, R.W.	1984	An evaluation of the synthetic pyrethroid cypermethrin in organic solvent and emulsion formulations. The International Research Group on Wood Preservation (working group III), paper prepared for the fifteenth annual meeting, Sweden, May 28 – June 1 1984. Building Research Establishment, UK, report no. IRG/WP/3290
B5.10.2/01	Fennert, E.M.	2006	Determination of the preventative action against *Reticulitermes santonesis* de Feytaud according to EN 118 (06/2005) after leaching procedure according to EN 84 (05/97). MPA Eberswalde, Materialprüfanstalt Brandenburg GmbH, Germany; report no. 32/05/8724/14, 32/05/8724/15, 32/05/8724/16; GLP not applicable, unpublished.
B5.10.2/02	Fennert, E.M.	2006	Determination of the toxic values against *Reticulitermes santonesis* de Feytaud according to EN 117 (06/2005) after evaporative ageing procedure according to EN 73 (04/90). MPA Eberswalde, Materialprüfanstalt Brandenburg GmbH, Germany; report no. 32/05/8724/09. GLP not applicable, unpublished.
B5.10.2/03	Fennert, E.M.	2006	Determination of the toxic values against *Reticulitermes santonesis* de Feytaud according to EN 117 (06/2005) after leaching procedure according to EN 84 (05/97). MPA Eberswalde, Materialprüfanstalt Brandenburg GmbH, Germany; report no. 32/05/8724/10. GLP not applicable, unpublished.
B5.10.2/04	Fennert, E.M.	2006	Determination of the preventative action against *Reticulitermes santonesis* de Feytaud according to EN 118 (06/2005) after leaching procedure according to EN 84 (05/97). MPA Eberswalde, Materialprüfanstalt Brandenburg GmbH, Germany; report no. 32/05/8724/14, 32/05/8724/15, 32/05/8724/16; GLP not applicable, unpublished.
B5.10.2/05	Fennert, E.M.	2006	Determination of the toxic values against *Reticulitermes santonesis* de Feytaud according to EN 117 (06/2005) after evaporative ageing procedure according to EN 73 (04/90). MPA Eberswalde, Materialprüfanstalt Brandenburg GmbH, Germany; report no. 32/05/8724/09. GLP not applicable, unpublished.

Study Summary / Annex point	Author	Year	Reference
B5.10.2/06	Fennert, E.M.	2006	Determination of the toxic values against *Reticulitermes santonesis* de Feytaud according to EN 117 (06/2005) after leaching procedure according to EN 84 (05/97). MPA Eberswalde, Materialprüfanstalt Brandenburg GmbH, Germany; report no. 32/05/8724/10. GLP not applicable, unpublished.
B5.10.2/07	Fennert, E.M.	2006	Determination of the preventative action against *Reticulitermes santonesis* de Feytaud according to EN 118 (06/2005) after leaching procedure according to EN 84 (05/97). MPA Eberswalde, Materialprüfanstalt Brandenburg GmbH, Germany; report no. 32/05/8724/14, 32/05/8724/15, 32/05/8724/16; GLP not applicable, unpublished.
B5.10.2/08	Fennert, E.M.	2006	Determination of the toxic values against *Reticulitermes santonesis* de Feytaud according to EN 117 (06/2005) after evaporative ageing procedure according to EN 73 (04/90). MPA Eberswalde, Materialprüfanstalt Brandenburg GmbH, Germany; report no. 32/05/8724/09. GLP not applicable, unpublished.

Section A5.2/01 Efficacy Data
Annex Point IIA-V.5.2 **House longhorn beetle (*Hylotrupes bajulus*)**

Official use only

1 REFERENCE

1.1	**Reference**	Anon. (1981); Determination of toxic values against *Hylotrupes bajulus* larvae; Building Research Establishment, Princes Risborough Laboratory, UK; report no. 80/11 (CYP/E28), 17 March 1981, (unpublished). Dates of work: 7th November 1980 (impregnation) – 12 March 1981 (examination).
1.2	**Data protection**	No
1.2.1	Data owner	Not applicable
1.2.2		
1.2.3	Criteria for data protection	No data protection claimed
1.3	**Guideline study**	Yes, EN 47
1.4	**Deviations**	No

2 METHOD

2.1	**Test Substance**	Cypermethrin (active substance)
2.1.1	Lot number	Not mentioned in report
2.1.2	Specification	Source of test material was Mitchell Cotts Chemicals
2.1.3	Description	Amber viscous liquid
2.1.4	Purity	Not mentioned in report
2.1.5	Method of analysis	No analysis of a.s. performed
2.2	**Reference substance**	Cypermethrin was tested by BRE alongside Permethrin and Decamethrin
2.2.1	Method of analysis for reference substance	No analysis performed
2.3	**Testing procedure**	
2.3.1	Test organism	*Hylotrupes bajulus* (category 1 – freshly hatched egg larvae)
2.3.2	Test system	Impregnated wood blocks (Scots Pine sapwood) were exposed to the test organism. Freshly hatched larvae were introduced into pre-drilled holes in the prepared blocks and survival determined after 12 weeks with the aid of autoradiographs.
2.3.3	Application of TS	Vacuum-pressure treatment (wood impregnation) performed on 7th November 1980.
2.3.4	Concentrations tested	0.00004, 0.0002, 0.001 % m/m Cypermethrin in solvent (Toluene). Mean retention: 0.22, 1.11, 5.70 g/m^3
2.3.5	Test conditions	After impregnation, wood blocks were allowed to air dry. No artificial ageing was performed.

Section A5.2/01 **Efficacy Data**
Annex Point IIA-V.5.2 **House longhorn beetle (*Hylotrupes bajulus*)**

2.3.6	Duration of the test / Exposure time	Date of impregnation: 07/11/1980 Date larvae introduced: 12/12/1980 Date of examination: 12/03/1981 Wood blocks were therefore exposed to the test organism for 12 weeks
2.3.7	Number of replicates performed	Not specified in report.
2.3.8	Controls	Yes, untreated and solvent controls were performed
2.4	**Examination**	
2.4.1	Effect investigated	Mortality of larvae
2.4.2	Method for recording / scoring of the effect	Counting of live or dead larvae (with and without tunnelling). Live larvae assessed using autoradiographs.
2.4.3	Intervals of examination	Autoradiographs were used at 4 weeks to assess the presence of live larvae in the wood blocks. Since all test concentrations showed live larvae were present, the wood blocks were subjected to the full 12 week incubation.
2.4.4	Statistics	Not performed
2.4.5	Post monitoring of the test organism	No
		3 RESULTS
3.1	**Efficacy**	
3.1.1	Dose/Efficacy curve	Not applicable
3.1.2	Begin and duration of effects	Not applicable
3.1.3	Observed effects in the post monitoring phase	Not applicable
3.2	**Effects against organisms or objects to be protected**	Mortality of larvae
3.3	**Other effects**	None
3.4	**Efficacy of the reference substance**	See Table A5_2-01
3.5	**Tabular and/or graphical presentation of the summarised results**	See Table A5_2-01
3.6	**Efficacy limiting factors**	

Section A5.2/01 **Efficacy Data**
Annex Point IIA-V.5.2 **House longhorn beetle (*Hylotrupes bajulus*)**

3.6.1	Occurrences of resistances	Not reported
3.6.2	Other limiting factors	Not reported

4 RELEVANCE OF THE RESULTS COMPARED TO FIELD CONDITIONS

4.1	**Reasons for laboratory testing**	Laboratory test was performed according to the accepted industry guideline European Standard no. EN 47
4.2	**Intended actual scale of biocide application**	Not applicable, test was performed on the active substance cypermethrin.
4.3	**Relevance compared to field conditions**	
4.3.1	Application method	Application method (vacuum-pressure) is comparable with industrial pre-treatment of wood. However in this instance the test was performed with the active substance in a solvent rather than a formulated product.
4.3.2	Test organism	Identical to target organism found in field conditions.
4.3.3	Observed effect	The toxic value for Cypermethrin was found to be 1.11 – 5.70 g/m^3
4.4	**Relevance for read-across**	This test demonstrates the efficacy of the active substance cypermethrin when applied as an industrial pre-treatment (vacuum-pressure).

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Scots pine sapwood blocks were impregnated with the active substance cypermethrin at three different concentrations in toluene. After a period of 30 days, larvae (category 1, freshly hatched) of the test organism *Hylotrupes bajulus* were introduced into pre-drilled holes in the test blocks and the survival rate determined after 12 weeks.
5.2	**Reliability**	Test results can be considered reliable as the method used was an accepted industry standard (EN 47) and was performed at an established facility.
5.3	**Assessment of efficacy, data analysis and interpretation**	Toxic value of Cypermethrin was found to be 1.11 – 5.70 g/m3
5.4	**Conclusion**	Cypermethrin showed acceptable efficacy against the larvae of house longhorn beetle when applied as a vacuum-pressure treatment
5.5	**Proposed efficacy specification**	See point 5.3 above

Section A5.2/01 **Efficacy Data**
Annex Point IIA-V.5.2 **House longhorn beetle (*Hylotrupes bajulus*)**

Evaluation by Competent Authorities

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

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	**16/09/2007**
Comments	**Test carried out according to a superseded version of EN standards - ACCEPTED**
Summary and conclusion	**Reliability 1** **The test report supports the applicant conclusion**

COMMENTS FROM ... (specify)

Date	Give date of comments submitted
Comments	Discuss if deviating from view of rapporteur member state
Summary and conclusion	Discuss if deviating from view of rapporteur member state

Table A5_2-01 Results of larvae assessment (12 weeks)

Concentration tested (% m/m)	Mass of solution absorbed per sample (g)			Mean retention (g/m3)	Number of larvae			
	Min.	Mean	Max.		Dead, without tunnelling	Dead, with tunnelling	Live	Not recovered
0.00004	9.76	10.41	11.15	0.22	11	2	16	1
0.0002	9.94	10.41	10.76	1.11	16	4	5	5
0.001	10.30	10.68	10.99	5.70	29	1	0	0
Solvent control	9.85	10.15	10.41	-	0	2	25	3
Untreated	-	-	-	-	0	1	24	5

Section A5.2/02 Efficacy Data
Annex Point IIA-V.5.2 **House longhorn beetle (*Hylotrupes bajulus*)**

Official use only

1 REFERENCE

1.1	**Reference**	Anon. (1981); Determination of toxic values against *Hylotrupes bajulus* larvae; Building Research Establishment, Princes Risborough Laboratory, UK; report no. 80/12-15 (CYP/E28), 17 March 1981, (unpublished). Dates of work: 5th November 1980 (impregnation) – 12 March 1981 (examination)
1.2	**Data protection**	No
1.2.1	Data owner	Not applicable
1.2.2		
1.2.3	Criteria for data protection	No data protection claimed
1.3	**Guideline study**	Yes, EN 47
1.4	**Deviations**	No

2 METHOD

2.1	**Test Substance**	Cypermethrin (active substance)
2.1.1	Lot number	Not mentioned in report
2.1.2	Specification	Source of test material was Mitchell Cotts Chemicals
2.1.3	Description	Amber viscous liquid
2.1.4	Purity	Not mentioned in report
2.1.5	Method of analysis	No analysis of a.s. performed
2.2	**Reference substance**	Cypermethrin was tested by BRE alongside Permethrin and Deltamethrin
2.2.1	Method of analysis for reference substance	No analysis performed
2.3	**Testing procedure**	
2.3.1	Test organism	*Hylotrupes bajulus* (category 2, 50-150 mg)
2.3.2	Test system	Impregnated wood blocks (Scots Pine sapwood) were exposed to the test organism. Freshly hatched larvae were introduced into pre-drilled holes in the prepared blocks and survival determined after 12 weeks.
2.3.3	Application of TS	Vacuum-pressure treatment (wood impregnation) performed on 5th November 1980.
2.3.4	Concentrations tested	0.000625, 0.00125, 0.0025 % m/m Cypermethrin in solvent (Toluene). Mean retention: 3.30, 6.70, 13.35 g/m^3
2.3.5	Test conditions	After impregnation, wood blocks were allowed to air dry. No artificial ageing was performed.

Section A5.2/02 **Efficacy Data**
Annex Point IIA-V.5.2 **House longhorn beetle (*Hylotrupes bajulus*)**

2.3.6	Duration of the test / Exposure time	Date of impregnation: 05/11/1980 Date larvae introduced: 18/12/1980 Date of examination: 12/03/1981 Wood blocks were therefore exposed to the test organism for 12 weeks
2.3.7	Number of replicates performed	Not specified in report.
2.3.8	Controls	Yes, untreated and solvent controls were performed
2.4	**Examination**	
2.4.1	Effect investigated	Mortality of larvae
2.4.2	Method for recording / scoring of the effect	Counting of live or dead larvae (with and without tunnelling).
2.4.3	Intervals of examination	Autoradiographs were used prior to assessment to determine the presence of live larvae in the wood blocks.
2.4.4	Statistics	Not performed
2.4.5	Post monitoring of the test organism	No
		3 RESULTS
3.1	**Efficacy**	
3.1.1	Dose/Efficacy curve	Not applicable
3.1.2	Begin and duration of effects	Not applicable
3.1.3	Observed effects in the post monitoring phase	Not applicable
3.2	**Effects against organisms or objects to be protected**	Mortality of larvae
3.3	**Other effects**	None
3.4	**Efficacy of the reference substance**	See Table A5_2-02
3.5	**Tabular and/or graphical presentation of the summarised results**	See Table A5_2-02
3.6	**Efficacy limiting factors**	
3.6.1	Occurrences of resistances	Not reported

Section A5.2/02 **Efficacy Data**
Annex Point IIA-V.5.2 **House longhorn beetle (*Hylotrupes bajulus*)**

3.6.2	Other limiting factors	Not reported

4 RELEVANCE OF THE RESULTS COMPARED TO FIELD CONDITIONS

4.1	**Reasons for laboratory testing**	Laboratory test was performed according to the accepted industry guideline European Standard no. EN 47
4.2	**Intended actual scale of biocide application**	Not applicable, test was performed on the active substance cypermethrin.
4.3	**Relevance compared to field conditions**	
4.3.1	Application method	Application method (vacuum-pressure) is comparable with industrial pre-treatment of wood. However in this instance the test was performed with the active substance in a solvent rather than a formulated product.
4.3.2	Test organism	Identical to target organism found in field conditions.
4.3.3	Observed effect	The toxic value for Cypermethrin was found to be 3.3 – 6.7 g/m^3
4.4	**Relevance for read-across**	This test demonstrates the efficacy of the active substance cypermethrin when applied as an industrial pre-treatment (vacuum-pressure).

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Scots pine sapwood blocks were impregnated with the active substance cypermethrin at three different concentrations in toluene. After a period of 43 days, larvae (category 2, 50-150 mg) of the test organism *Hylotrupes bajulus* were introduced into pre-drilled holes in the test blocks and the survival rate determined after 12 weeks.
5.2	**Reliability**	Test results can be considered reliable as the method used was an accepted industry standard (EN 47) and was performed at an established facility.
5.3	**Assessment of efficacy, data analysis and interpretation**	Toxic value of Cypermethrin was found to be 6.7 - 13.35 g/m^3
5.4	**Conclusion**	Cypermethrin showed acceptable efficacy against the larvae of house longhorn beetle when applied as a vacuum-pressure treatment.
5.5	**Proposed efficacy specification**	See point 5.3 above

Section A5.2/02 **Efficacy Data**
Annex Point IIA-V.5.2 **House longhorn beetle (*Hylotrupes bajulus*)**

Evaluation by Competent Authorities

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

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	**16/09/2007**
Comments	**Test carried out according to a superseded version of EN standards - ACCEPTED**
Summary and conclusion	**Reliability 1** **The test report supports the applicant conclusion**

COMMENTS FROM ... (specify)

Date	Give date of comments submitted
Comments	Discuss if deviating from view of rapporteur member state
Summary and conclusion	Discuss if deviating from view of rapporteur member state

Table A5_2-02 Results of larvae assessment (12 weeks)

Concentration tested (% m/m)	Mass of solution absorbed per sample (g)			Mean retention (g/m3)	Number of larvae			
	Min.	Mean	Max.		Dead, without tunnelling	Dead, with tunnelling	Live	Not recovered
0.000625	8.90	9.85	10.24	3.30	3	3*	3	0
0.00125	9.36	10.05	10.55	6.70	8	1	1	0
0.0025	8.92	10.01	10.55	13.35	9	1	0	0
Solvent control	9.45	10.02	10.44	-	0	0	10	0
Untreated	-	-	-	-	0	0	10	0

* plus 1 moribund

Section A5.2/03 Efficacy Data
Annex Point IIA-V.5.2 **Furniture beetle (*Anobium punctatum*)**

Official use only

1 REFERENCE

1.1	**Reference**	Anon. (1980); Determination of toxic values against *Anobium punctatum* larvae; Building Research Establishment, Princes Risborough Laboratory, UK; (CYP/E28), 18 November 1980, (unpublished). Dates of work: 15th October 1979 (impregnation) – 18 November 1980 (examination)
1.2	**Data protection**	No
1.2.1	Data owner	Not applicable
1.2.2		
1.2.3	Criteria for data protection	No data protection claimed
1.3	**Guideline study**	Yes, EN 21
1.4	**Deviations**	No

2 METHOD

2.1	**Test Substance**	Cypermethrin (active substance)
2.1.1	Lot number	Not mentioned in report
2.1.2	Specification	Source of test material was Mitchell Cotts Chemicals
2.1.3	Description	Amber viscous liquid
2.1.4	Purity	Not mentioned in report
2.1.5	Method of analysis	No analysis of a.s. performed
2.2	**Reference substance**	Cypermethrin was tested by BRE alongside Permethrin and Decamethrin
2.2.1	Method of analysis for reference substance	No analysis performed
2.3	**Testing procedure**	
2.3.1	Test organism	*Anobium punctatum* larvae (3-5 mg)
2.3.2	Test system	Impregnated wood blocks (Scots Pine sapwood) were exposed to the test organism. Freshly hatched larvae were introduced into pre-drilled holes in the prepared blocks and survival determined after 12 months.
2.3.3	Application of TS	Vacuum-pressure treatment (wood impregnation) performed on 15th October 1979.
2.3.4	Concentrations tested	0.002, 0.004, 0.008 % m/m Cypermethrin in solvent (Toluene). Mean retention: 9.7, 20.3, 39.3 g/m^3
2.3.5	Test conditions	After impregnation, wood blocks were allowed to air dry. No artificial ageing was performed.

Section A5.2/03 **Efficacy Data**
Annex Point IIA-V.5.2 **Furniture beetle (*Anobium punctatum*)**

2.3.6	Duration of the test / Exposure time	Date of impregnation: 15/10/79 Date larvae introduced: 30/11/79 Date of examination: 18/11/80 Wood blocks were therefore exposed to the test organism for 12 months
2.3.7	Number of replicates performed	Not specified in report.
2.3.8	Controls	Yes, untreated and solvent controls were performed
2.4	**Examination**	
2.4.1	Effect investigated	Mortality of larvae
2.4.2	Method for recording / scoring of the effect	Counting of live or dead larvae (with and without tunnelling).
2.4.3	Intervals of examination	Autoradiographs were used prior to final assessment and after 6 months to determine the presence of live larvae in the wood blocks.
2.4.4	Statistics	Not performed
2.4.5	Post monitoring of the test organism	No
		3 RESULTS
3.1	**Efficacy**	
3.1.1	Dose/Efficacy curve	Not applicable
3.1.2	Begin and duration of effects	Not applicable
3.1.3	Observed effects in the post monitoring phase	Not applicable
3.2	**Effects against organisms or objects to be protected**	Mortality of larvae
3.3	**Other effects**	None
3.4	**Efficacy of the reference substance**	See Table A5_2-03
3.5	**Tabular and/or graphical presentation of the summarised results**	See Table A5_2-03
3.6	**Efficacy limiting factors**	
3.6.1	Occurrences of resistances	Not reported

Section A5.2/03 **Efficacy Data**
Annex Point IIA-V.5.2 **Furniture beetle (*Anobium punctatum*)**

3.6.2	Other limiting factors	Not reported

4 RELEVANCE OF THE RESULTS COMPARED TO FIELD CONDITIONS

4.1	**Reasons for laboratory testing**	Laboratory test was performed according to the accepted industry guideline European Standard no. EN 21
4.2	**Intended actual scale of biocide application**	Not applicable, test was performed on the active substance cypermethrin.
4.3	**Relevance compared to field conditions**	
4.3.1	Application method	Application method (vacuum-pressure) is comparable with industrial pre-treatment of wood. However in this instance the test was performed with the active substance in a solvent rather than a formulated product.
4.3.2	Test organism	Identical to target organism found in field conditions.
4.3.3	Observed effect	The toxic value for Cypermethrin was found to be 20.3 – 39.3 g/m^3
4.4	**Relevance for read-across**	This test demonstrates the efficacy of the active substance cypermethrin when applied as an industrial pre-treatment (vacuum-pressure).

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Scots pine sapwood blocks were impregnated with the active substance cypermethrin at three different concentrations in toluene. After a period of 46 days, larvae (3-5mg) of the test organism *Anobium punctatum* were introduced into pre-drilled holes in the test blocks and the survival rate determined after 12 months.
5.2	**Reliability**	Test results can be considered reliable as the method used was an accepted industry standard (EN 21) and was performed at an established facility.
5.3	**Assessment of efficacy, data analysis and interpretation**	Toxic value of Cypermethrin was found to be 9.7 – 39.3 g/m^3
5.4	**Conclusion**	Cypermethrin showed acceptable efficacy against the larvae of the furniture beetle when applied as a vacuum-pressure treatment.
5.5	**Proposed efficacy specification**	See point 5.3 above

Section A5.2/03 **Efficacy Data**
Annex Point IIA-V.5.2 **Furniture beetle (*Anobium punctatum*)**

Evaluation by Competent Authorities

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

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	**30/09/2007**
Comments	**Test carried out according to a superseded version of EN standards – ACCEPTED** **Experimental toxic value rather high as compared to EN 47 and other EN 21 results.**
Summary and conclusion	**Reliability 1** **The test report supports the applicant conclusions**

COMMENTS FROM ... (specify)

Date	Give date of comments submitted
Comments	Discuss if deviating from view of rapporteur member state
Summary and conclusion	Discuss if deviating from view of rapporteur member state

Table A5_2-03 Results of larvae assessment (12 months)

Concentration tested (% m/m)	Mass of solution absorbed per sample (g)			Mean retention (g/m3)	Number of larvae				
	Min.	Mean	Max.		Live at 6 months*	Dead, without tunnelling	Dead, with tunnelling	Live	Not recovered
0.002	8.15	9.08	9.44	9.7	20	19	5	18	8
0.004	9.03	9.50	9.95	20.3	17	19	22	8	1
0.008	7.61	9.22	10.13	39.3	0	49	1	0	0
Solvent control	8.66	9.65	10.49	-	41	0	1	39	10[1]
Untreated	-	-	-	-	39	0	1	32	17[2]

* estimated by autoradiograph at 6 months
[1] 4 emerged as beetles
[2] 7 emerged as beetles

Section A5.2/04 Efficacy Data
Annex Point IIA-V.5.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	Read S.J., Berry, R.W. (1984); An evaluation of the synthetic pyrethroid cypermethrin in organic solvent and emulsion formulations. The International Research Group on Wood Preservation (working group III), paper prepared for the fifteenth annual meeting, Sweden, May 28 – June 1 1984. Building Research Establishment, UK, report no. IRG/WP/3290, 21 March 1984 (published).	
1.2	**Data protection**	No	
1.2.1	Data owner	Not applicable, published report.	
1.2.2			
1.2.3	Criteria for data protection	No data protection claimed.	
1.3	**Guideline study**	Yes, European Standards	
		EN 21, EN 49, EN 48 (*Anobium* spp.)	
		EN 46, EN 47, EN 22 (*Hylotrupes* spp.)	
		EN117, EN 118 (*Reticulitermes* spp.)	
		EN 20 (*Lyctus* spp.)	
1.4	**Deviations**	Yes, EN 46 and EN 20 were modified to British Standard BS 5761 (ageing procedure)	
		2 METHOD	
2.1	**Test Substance**	Cypermethrin (organic solvent and emulsion formulations)	
2.1.1	Lot number	Not mentioned in report	
2.1.2	Specification	Not mentioned in report	
2.1.3	Description	Not mentioned in report	
2.1.4	Purity	Not mentioned in report	
2.1.5	Method of analysis	No analysis performed	
2.2	**Reference substance**	Not included, however results were compared to known toxic values for Permethrin and γ-HCH.	
2.2.1	Method of analysis for reference substance	No analysis performed	
2.3	**Testing procedure**		
2.3.1	Test organisms	Furniture beetle (*Anobium punctatum*)	
		House longhorn beetle (*Hylotrupes bajulus*)	
		Powder post beetle (*Lyctus brunneus*)	
		Termites (*Reticulitermes spp.)*	

Section A5.2/04 **Efficacy Data**
Annex Point IIA-V.5.2

2.3.2	Test system	Bioassay tests were carried out according to European standards. Assessment of preventative efficacy against *Anobium* (EN 21 and EN 49), *Hylotrupes* (EN 46 and EN 47), *Reticulitermes* (EN 117, EN 118) and *Lyctus (*EN 20). Assessment of eradicant efficacy was also carried out against *Anobium* (EN22) and *Hylotrupes* (EN 22). Emergence tests were also performed with *Anobium* (test described by Berry, 1982).
2.3.3	Application of TS	Vacuum-pressure treatment (wood impregnation) or surface treatment . Accelerated ageing of the test samples was performed according to British standard BS 5761.
2.3.4	Concentrations tested	See Tables A5_2_04-1 to A5_2_04-4
2.3.5	Test conditions	Tests were performed with and without artificial ageing.
2.3.6	Duration of the test / Exposure time	Not specified in report
2.3.7	Number of replicates performed	Not specified in report.
2.3.8	Controls	Not specified in report
2.4	**Examination**	
2.4.1	Effect investigated	Mortality of larvae, number of emergent adults (Anobium emergence test only).
2.4.2	Method for recording / scoring of the effect	Counting of live or dead larvae.
2.4.3	Intervals of examination	Not specified in report.
2.4.4	Statistics	Not performed
2.4.5	Post monitoring of the test organism	No

3 RESULTS

3.1	**Efficacy**	
3.1.1	Dose/Efficacy curve	Not applicable
3.1.2	Begin and duration of effects	Ageing procedures showed that cypermethrin had considerable resistance to standardised accelerated evaporative ageing. In the *Anobium* emergence tests, 0.1 % cypermethrin emulsion prevented emergence for up to 5 years.
3.1.3	Observed effects in the post monitoring phase	Not applicable

Section A5.2/04 Efficacy Data
Annex Point IIA-V.5.2

3.2	**Effects against organisms or objects to be protected**	Mortality.
3.3	**Other effects**	None
3.4	**Efficacy of the reference substance**	In general, cypermethrin was found to be twice as effective as Permethrin (based on known toxic values) and comparable to γ-HCH, depending on the insect species, life cycle stage and size of larvae. Against mature *Anobium* larvae, cypermethrin was approximately equal in effectiveness to γ-HCH but four times more effective against egg larvae. Against emergent *Anobium*, 0.1% cypermethrin was more effective than a solution of γ-HCH ten times stronger. Against *Hylotrupes* larvae, cypermethrin was four times more active and a solution fifty times less than that used commercially for γ-HCH was found to be effective as a surface spray against *Lyctus*.
3.5	**Tabular and/or graphical presentation of the summarised results**	See Tables A5_2_04-1 to A5_2_04-4
3.6	**Efficacy limiting factors**	
3.6.1	Occurrences of resistances	Not reported
3.6.2	Other limiting factors	Not reported

4 RELEVANCE OF THE RESULTS COMPARED TO FIELD CONDITIONS

4.1	**Reasons for laboratory testing**	Laboratory test was performed according to the accepted European Standards to compare potential efficacy of cypermethrin at various concentrations as both an emulsion and organic solvent formulation.
4.2	**Intended actual scale of biocide application**	From this study it can be seen that cypermethrin has considerable potential as a wood preservative for both preventative (impregnation and surface applications) and curative treatments.
4.3	**Relevance compared to field conditions**	
4.3.1	Application method	Application methods were comparable with industrial pre-treatment and remedial applications.
4.3.2	Test organism	Identical to target organisms found in field conditions.
4.3.3	Observed effect	These laboratory investigations demonstrated the efficacy of the active ingredient cypermethrin against wood destroying insects when formulated as an emulsion or organic solvent.

Section A5.2/04 Efficacy Data
Annex Point IIA-V.5.2

4.4	**Relevance for read-across**	In general, the test results show effective action against all insects tested and comparable to other active substances used in wood treatment formulations. Against *Reticulitermes*, surface applications using emulsion formulation performed well with 0.1% cypermethrin being adequate (may need to be higher for larger termite species). Only low levels of cypermethrin were required to kill mature *Hylotrupes* larvae (6.7-13.4 g/m^3) with higher levels required for *Anobium*. In the eradicant test for *Anobium*, emulsion formulations were less effective than organic solvent formulations. However, infestations of this species are rarely sufficient to warrant curative treatment, therefore good control of emergent adults is usually sufficient. Against *Hylotrupes*, emulsion formulations appeared to perform better in the eradicant tests as larvae tend to tunnel closer to the surface. All cypermethrin formulations performed well in the *Anobium* emergence tests, even with 5 years ageing, and were considered superior to treatment with other active substances previously tested.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Bioassay tests were carried out against a range of wood boring insects according to EN standards (*Lyctus, Hylotrupes, Reticulitermes* and *Anobium Spp.*) A range of cypermethrin concentrations were tested, using both emulsion and organic solvent formulations.
5.2	**Reliability**	Test results can be considered reliable as the methods used are accepted industry standards and were performed at an established facility.
5.3	**Assessment of efficacy, data analysis and interpretation**	Loadings required for preventative pre-treatments were considered to be: 0.67-1.54 g/m^3 against *Anobium* egg larvae 1.1-5.7 g/m^3 against *Hylotrupes* egg larvae 49.7-100 g/m^3 against *Reticulitermes* For brush treatments, 0.1% cypermethrin was to adequate against *Reticulitermes*. For dip applications against *Lyctus*, 0.0021-0.0046 g/m^2 was found to be effective. For curative applications, 0.1% cypermethrin appeared to be adequate in organic solvent formulations. For preventing emergence of adult *Anobium*, 0.1% cypermethrin in organic solvent and emulsion formulations was also found to be an effective treatment.
5.4	**Conclusion**	Cypermethrin showed acceptable efficacy against all species of wood destroying insects tested and in some cases was found to be considerably more effective at lower concentrations compared to other active substances used commercially for wood treatment.
5.5	**Proposed efficacy specification**	See point 5.3 above

Section A5.2/04 **Efficacy Data**
Annex Point IIA-V.5.2

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	**16/09/2007**
Comments	**Test carried out according to superseded versions of EN standards - ACCEPTED**
Summary and conclusion	**Reliability 1**
	The test report supports the applicant conclusions
	COMMENTS FROM ... (specify)
Date	Give date of comments submitted
Comments	Discuss if deviating from view of rapporteur member state
Summary and conclusion	Discuss if deviating from view of rapporteur member state

Table A5_2-04-1 Results of larvae assessment – Preventative Surface Treatment

Test	Insect	Formulation type	Conc. tested (%)	App. rate	Ageing	Toxic value
EN 46	Hylotrupes (egg larvae)	OS	0.000125-0.001	160 g/m^2	-	0.00025-0.0005 %
EN 46	Hylotrupes (egg larvae)	OS	0.000125-0.001	160 g/m^2	12 weeks	0.00025-0.0005 %
EN 20	Lyctus (egg laying)	Emulsion	0.00125-0.02	1 min. dip	-	0.0021-0.0046 g/m^2 (0.0025-0.005%)
EN 20	Lyctus (egg laying)	Emulsion	0.01-0.1	100-200 ml/ m^2	12 weeks	No infestation
EN 118	Reticulitermes	Emulsion	0.1	300 ml/ m^2	-	Grade 0 (6 replicates)
EN 118	Reticulitermes	Emulsion	0.1	300 ml/ m^2	12 weeks	Grade 0 (4 replicates) Grade 1 (2 replicates)
EN 118	Reticulitermes	Emulsion	0.1	300 ml/ m^2	-	Grade 0 (2 replicates) Grade 1 (4 replicates)
EN 118	Reticulitermes	Emulsion	0.1	300 ml/ m^2	12 weeks	Grade 0 (2 replicates) Grade 1 (3replicates) Grade 2 (1 replicate)
EN 118	Reticulitermes	OS	0.1	300 ml/ m^2	-	Grade 0 (6 replicates)
EN 118	Reticulitermes	OS	0.1	300 ml/ m^2	12 weeks	Grade 0 (5 replicates) Grade 1 (1 replicate)
EN 118	Reticulitermes	OS	0.05	300 ml/ m^2	-	Grade 0 (2 replicates) Grade 1 (4 replicates)
EN 118	Reticulitermes	OS	0.1	300 ml/ m^2	-	Grade 0 (5 replicates) Grade 1 (1 replicate)

OS = Organic solvent

Table A5_2-04-2 Results of larvae assessment – Preventative Impregnation Treatment

Test	Insect	Formulation type	Mean Loading (g/m^3)	Ageing	Toxic values[1] (g/m^3)
EN 49	Anobium (egg laying)	OS	0.35-5.60	-	0.76-1.59
EN 49	Anobium (egg laying)	OS	0.35-5.60	12 weeks	0.67-1.54
EN 21	Anobium (mature larvae)	OS	9.7-39.3	-	20.3-39.3
EN 47	Hylotrupes (egg larvae)	OS	0.2-5.7	-	1.1-5.7
EN 47	Hylotrupes (mature larvae)	OS	3.3-13.4	-	6.7-13.4
117	Reticulitermes	OS	24.6-414.4	-	49.7-100

[1]Highest loading permitting survival, lowest loading preventing survival

OS = Organic solvent

Table A5_2-04-3 Results of larvae assessment – Remedial Treatment

Test	Insect	Formulation type	Conc. (%)[1]	Mean mortality (%)
EN 48	Anobium	Emulsion	0.1	23.6
EN 48	Anobium	Emulsion	0.1	36.1
EN 48	Anobium	OS (Shellsol AB)	0.1	75.0
EN 48	Anobium	OS (odourless kerosene)	0.1	94.4
EN 48	Anobium	OS (odourless kerosene)	0.05	93.1
EN 48	Anobium	OS (odourless kerosene)	0.1	93.1
EN 22	Hylotrupes	Emulsion	0.1	75.0
EN 22	Hylotrupes	Emulsion	0.1	77.1
EN 22	Hylotrupes	OS (Shellsol AB)	0.1	93.8
EN 22	Hylotrupes	OS (odourless kerosene)	0.05	66.7
EN 22	Hylotrupes	OS (odourless kerosene)	0.1	87.5

[1]Application rate 300 ml/m^2 in all cases

OS = Organic Solvent

Table A5_2-04-4 Remedial emergence tests – *Anobium* Spp.

Ageing[1]	Formulation type	Conc. (%)	App. Rate (ml/m^2)	Attempted emergence (%)	Failed emergence (%)
-	Emulsion A	0.1	300	36	100
-	Emulsion B	0.1	300	35	100
-	OS (Shellsol AB)	0.1	300	39	100
12 weeks	Emulsion A	0.1	300	35	100
12 weeks	Emulsion B	0.1	300	47	100
12 weeks	OS (Shellsol AB)	0.1	300	48	88
26 months	OS (Shellsol E)	0.1	300	68	100
26 months	OS (Shellsol E)	0.2	300	70	100
62 months	Emulsion	0.1	200	67.5	100
62 months	Emulsion	0.1	200	75	100
62 months	OS (odourless kerosene)	0.1	200	70	96

[1]Ageing for 12 weeks according to BS 5761, ageing to 26 and 62 months in unheated, ventilated storage building

Section A6.1.1 Acute Oral Toxicity - Rat

Annex Point IIA 6.1

Rat, cypermethrin, oral LD50

			Official use only
		1 REFERENCE	
1.1	**Reference**	Kobel, W (1984); Acute Oral LD50 in the Rat of CGA 55186 Tech. (cypermethrin) – (administration in oily medium); Ciba-Geigy Ltd, report No.:840042 (CYP/T82b), 9 April 1984 (unpublished) Dates of work: 13 February 1984 – 2 May 1984	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Chimac-Agriphar s.a.	
1.2.2			
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I authorisation	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No Existing study partially conforming to 92/69/EEC (OECD 401)	
2.2	**GLP**	No GLP was not compulsory at the time the study was performed	
2.3	**Deviations**	Yes Protocol partially conforms to OECD 401 (EC method B1) but with limited enquiries	
		3 MATERIALS AND METHODS	
3.1	**Test material**	CGA 55186 tech (cypermethrin cis:trans/40:60)	
3.1.1	Lot/Batch number	307046	
3.1.2	Specification	Deviating from specification given in section 2 as follows	
3.1.2.1	Description	Liquid	
3.1.2.2	Purity	92.6%	
3.1.2.3	Stability	Guaranteed by original sponsor (Ciba-Geigy Ltd)	X
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Tif:RAIf (SPF), F3-crosses of RII 1/Tiff x RII 2/Tif	
3.2.3	Source	Ciba Geigy Ltd, Tierfarm 4334 Sisseln, Switzerland	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	7-8 weeks, 162-225 g	
3.2.6	Number of animals per group	5 males, 5 females	
3.2.7	Control animals	No	

Section A6.1.1 **Annex Point IIA 6.1**		**Acute Oral Toxicity - Rat** **Rat, cypermethrin, oral LD50**	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Postexposure period	14 days or until all symptoms disappeared	
		Oral	
3.3.2	Type	Gavage	
3.3.3	Concentration	Gavage: 5000, 2500, 1200, 600, 300 mg/kg bw food consumption per day - ad libitum	
3.3.4	Vehicle	Arachis oil Ph.H.VI Siegfried AG	
3.3.5	Concentration in vehicle	As 3.3.3	
3.3.6	Total volume applied	10 ml/kg bw	
3.3.7	Controls	None	
3.4	**Examinations**	Mortality recorded twice daily (once daily on weekends), clinical observations daily, body weight recorded on days 1, 7, 14 and at death. Gross necropsy at death and all survivors at the end of the observation period.	
3.5	**Method of determination of LD_{50}**	Logit method (J. Berkson, J.Am.Stat. Ass. 39. 357-65, 1944): LD50 including the 95% confidence limit	
3.6	**Further remarks**	-	
		4 RESULTS AND DISCUSSION	
4.1	**Clinical signs**	Dyspnoea, exophthalmus, ruffled fur and curved body position observed. Diarrhoea, tremor, tonic clonic convulsion, salivation, sedation and lateral/ventral body positions were also seen. Surviving animals recovered within 10 to 12 days.	X
4.2	**Pathology**	Autopsies showed no gross lesions in the three lowest dose groups (300, 600, and 1200 mg/kg bw groups). In the 2500 mg/kg bw group one female had nasal discharge. In the 5000 mg/kg bw group a reddish and mottled lung was found and one animal had a dilated small intestine.	
4.3	**Other**		
4.4	**LD_{50}**	LD50 in males 1732 (1027-2922) mg/kg bw LD50 in females 2150 (1342-4024) mg/kg bw LD50 in both sexes 1945 (1449-2676) mg/kg bw	

Section A6.1.1 **Acute Oral Toxicity - Rat**

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

		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Acute oral administration in rat with 14 day post-treatment observation based on OECD guideline 401.
5.2	**Results and discussion**	An LD50 of 1945 mg/kg (with 95% confidence limits) was calculated for both sexes.
5.3	**Conclusion**	
5.3.1	Reliability	2
5.3.2	Deficiencies	Yes Limited enquiries, however protocol was based on EC test method B1. Study has been previously evaluated under Directive 91/414/EC.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March, 2007.
Materials and Methods	The applicant's version is acceptable with the following amendment: 3.1.2.3 Stability: ... but data not shown.
Results and discussion	The applicant's version is adopted with the following amendments: Revised version **4.1 Clinical signs**: Dyspnoea, exophthalmus, ruffled fur and curved body position was observed in all test groups. Diarrhoea and tremor were also observed. Tonic clonic convulsion was observed from 600 mg/kg bw onwards. At higher concentrations also salivation, sedation and lateral/ventral body positions were observed. Surviving animals recovered within 10 to 12 days. Animals died during the first 3 days post-exposure.
Conclusion	LD50 in both sexes: 1945 (1449-2676) mg/kg bw LD50 in females: 2150 (1342-4024) mg/kg bw **LD50 in males: 1732 (1027-2922) mg/kg bw** LD50 males = 1732 mg/kg bw will be used for risk characterization purposes.
Reliability	*2*
Acceptability	acceptable (Although the study was performed before GLP and despite the limited enquiries, the protocol was based on EC test method B and is found acceptable.)
Remarks	

Section A6.1.1 Acute Oral Toxicity - Rat

Annex Point IIA 6.1

Rat, cypermethrin, oral LD50

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

Table A6_1_1. Table for Acute Oral Toxicity

Dose [mg/kg]	*Number of dead / number of investigated*	*Time of death (range)*	*Observations*
300	0/10	-	Dyspnoea, exophthalmus, ruffled fur, curved body position. All animals recovered by day 10.
600	0/10	-	Dyspnoea, exophthalmus, ruffled fur, diarrhoea, curved body position, convulsion. All animals recovered by day 11.
1200	1/10	Day 1 (post-exposure period)	Dyspnoea, exophthalmus, ruffled fur, ventral or curved body position, tremor, convulsion. All surviving animals recovered by day 10.
2500	7/10	Day 1 (post-exposure period)	Sedation, dyspnoea, salivation, ruffled fur, diahrroea, lateral or curved body position, convulsion. Surviving animals recovered by day 12.
5000	10/10	Days 1 to 3 (post-exposure period)	Dyspnoea, exophthalmus, salivation, ruffled fur, ventral, lateral or curved body position, tremor, convulsion.
LD_{50} value	1945 (1449-2676) mg/kg bw		

Section A6.1.1 Acute Oral Toxicity - Rat

Annex Point IIA 6.1 **Rat, neonatal and adult, two pyrethroids permethrin and Cypermethrin, acute oral toxicity**

			Official use only
		1 REFERENCE	
1.1	**Reference**	Cantalamessa, F (1993); Acute toxicity of two pyrethroids, permethrin and Cypermethrin, in neonatal and adult rats. Arch Toxicol (1993) 67: 510-513 (published)	
1.2	**Data protection**	No	
1.2.1	Data owner	Public domain literature	
1.2.2			
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No, a specific investigation to determine the relative toxicity of a type I and type II pyrethroid when administered on a single occasion by the oral route to neonatal and adult rats. The study was also designed to assess pyrethroid biotransformation through the use of drug metabolism inhibitors and the effects on Cypermethrin and permethrin toxicity.	
2.2	**GLP**	No	
2.3	**Deviations**	Not applicable.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Cypermethrin (type II pyrethroid) and permethrin (type I pyrethroid)	
3.1.1	Lot/Batch number	Technical grade Cypermethrin (NRDC 149) Technical grade permethrin (NRDC 143)	
3.1.2	Specification	Technical grade Cypermethrin – 62.8:37.2 *trans:cis*, 92.4% purity Technical grade permethrin – 75:25 *trans:cis*, 94% purity	
3.1.2.1	Description	No information	
3.1.2.2	Purity	92.4% or 94% as detailed above	
3.1.2.3	Stability	No information	X
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Wistar, bred in-house.	
3.2.3	Source	Bred in-house at Institute of Pharmacology, University of Camerino, Italy	
3.2.4	Sex	Male adults; male and female neonates	
3.2.5	Age/weight at study initiation	Adult age/weight details not provided Neonates of 8, 16 or 21 days of age used	
3.2.6	Number of animals per group	Ten animals per dose group	
3.2.7	Control animals	Yes	

Section A6.1.1 Acute Oral Toxicity - Rat

Annex Point IIA 6.1 **Rat, neonatal and adult, two pyrethroids permethrin and Cypermethrin, acute oral toxicity**

3.3	**Administration/ Exposure**	Oral
3.3.1	Postexposure period	No information provided
		Oral
3.3.2	Type	Gavage
3.3.3	Concentration	Dose levels not detailed in publication
3.3.4	Vehicle	Corn oil
3.3.5	Concentration in vehicle	Not specified
3.3.6	Total volume applied	5 ml/kg bw
3.3.7	Controls	Yes, vehicle dosed controls
3.4	**Examinations**	Mortality, clinical observations
3.5	**Method of determination of LD_{50}**	Thompson and Weil LD50 including the 95% confidence limits presented in point 4.4 below
3.6	**Further remarks**	

Section A6.1.1 **Acute Oral Toxicity - Rat**

Annex Point IIA 6.1 **Rat, neonatal and adult, two pyrethroids permethrin and Cypermethrin, acute oral toxicity**

4 RESULTS AND DISCUSSION

4.1	**Clinical signs**	Permethrin induced within 120-150 minutes of dosing, in neonates and adults, signs of hyperexcitability, aggressive behaviour and slight twitching at low doses and intense tremors, prostration and death at high doses, typical of a Type I pyrethroid T-syndrome. Cypermethrin elicited reactions in both adults and neonates (90-120 minutes after dosing) that included pawing and burrowing activity, facial licking and grooming at low doses and uncoordinated movements, coarse tremors, choreoathetosis, clonic seizure and death at high doses (typical for Type II CS syndrome for pyrethroid administration).	X
4.2	**Pathology**	No details of pathological examinations	
4.3	**Other**	A single oral administration of Cypermethrin and permethrin to neonatal and adult rats showed that Cypermethrin is more toxic than permethrin to adult and neonatal rats. It was noted that the sensitivity of rats to both test materials was greater the younger the animal. Use of a monooxygenase inhibitor or esterase inhibitor (pre-treatment of rats aged 8, 16 or 21days) did not cause any variation in the lethal effects of both test materials in neonatal rats. In the similarly pre-treated adult rats there was a significant increase in the toxicity of both test materials in adults treated with esterase inhibitors but no increase in toxicity after treatment with monooxygenase inhibitor. It is postulated that the greater sensitivity in neonates reflects incomplete development of the enzymes that catalyse pyrethroid metabolism in the liver. Ester hydrolysis is suggested as an important mechanism for pyrethroid detoxification in adults. The median lethal dose for Cypermethrin was in the range of 15-50 mg/kg for neonates and some five-fold higher for adults, 250 mg/kg bw. For permethrin the neonate range was notably higher than for Cypermethrin, circa 340-470 mg/kg bw and for the adults the median lethal dose was threefold higher than the weanling value. The toxicity and sensitivity of neonates in the 8-16 day range was markedly higher for Cypermethrin. The type I pyrethroid permethrin induced a typical T syndrome response after 120-150 minutes, whereas the type II Cypermethrin induced a CS response within 90-120 minutes. Both responses typically result in death. Profuse salivation was evident among the adult rats following Cypermethrin treatment, but not among the young pups (8 or 16 days old) and only sporadically among the weanlings (21 day old). Pre-treatment with TOPT and PB brought forward the onset of this response and also increased the intensity of the reaction. Pre-treatment with PB or TOPT did not produce a significant variation in lethality response in Cypermethrin treated neonates (8, 16 or 21 days) but mortality was significantly increased among the adults pre-treated with the esterase inhibitor, although a similar effect was not apparent for PB.	

Section A6.1.1 **Acute Oral Toxicity - Rat**

Annex Point IIA 6.1 **Rat, neonatal and adult, two pyrethroids permethrin and Cypermethrin, acute oral toxicity**

Pre-treatment of adult rats with TOPT similarly increased the percent mortality but the young rats (8, 16 or 21 days) were unaffected. Pre-treatment with PB had no effect on mortality for any of the adult or neonate rats.

4.4 **LD_{50}**

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

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

Median lethal doses for permethrin:

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

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 **Materials and methods**

Permethrin and Cypermethrin are two synthetic pyrethroid derivatives of natural pyrethrins. Both materials are neurotoxins (classified as type II– Cypermethrin or type I – permethrin, based on toxic signs observed in rats). Type I neurotoxic 'T' syndrome in rats and mice consists of aggressive sparring, increased sensitivity to external stimuli, fine tremors progressing to whole body tremor and prostration.

Type II syndrome ('CS') consists of pawing, burrowing, profuse salivation, coarse tremors progressing to choreoathetosis and clonic seizure.

Pyrethroids are rapidly metabolised in mammals. Toxicity and biotransformation of pyrethroids has been relatively well characterised in adult rats but less so in immature or neonate animals. Offspring may be at greater risk due to underdeveloped detoxification mechanisms, potentially resulting in adverse neurotoxicity if sufficient concentration reaches the nervous system.

Permethrin has relatively low mammalian toxicity – attributed to rapid metabolic detoxification and excretion – the *trans*-permethrin isomer is rapidly hydrolysed by esterase and the *cis* isomer more slowly.

The major route of biotransformation of the *trans*-isomer of Cypermethrin is ester cleavage, with the *trans* form hydrolysed more rapidly than the *cis* isomer, by microsomal carboxylesterase.

Drug metabolism in neonates tends to progress more slowly than in adults and drug metabolising enzymatic activity is less in newborns than adults. This study was designed to investigate the relative acute toxicity of two pyrethroids under similar conditions, comparing neonate and adult responses and assessing the importance of biotransformation through the use of metabolism inhibitors – tri-ortho-tolyl-phosphate (TOPT), an esterase inhibitor or piperonyl butoxide (PB) a monooxygenase inhibitor.

Section A6.1.1	**Acute Oral Toxicity - Rat**
Annex Point IIA 6.1	**Rat, neonatal and adult, two pyrethroids permethrin and Cypermethrin, acute oral toxicity**

The two test materials investigated were technical grade Cypermethrin (62.8:37.2 *trans:cis*, purity 92.4%) and technical grade permethrin (75:25 *trans:cis*, purity 94%).

Wistar rats, pups and adults were bred in-house, the pups were maintained with their dams until weaning at 21 days. Pups of both sexes were used in the study but only male adult rats were treated. Prior to the single oral treatment, the adults were fasted for 16 h, the pups of 8 days were removed from the dam for a period of one hour and those of 16 or 21 days of age were removed for two hours. Food was returned to the adults immediately after dosing and the pups were returned to the mothers immediately following treatment.

Ten animals per dose level were used to determine the LD_{50} values for the various age groups.

The test substances were both dissolved in corn oil and administered in a dose volume of 5 ml/kg bw. Controls received the vehicle alone.

Pre-treatment groups involved intraperitoneal administration of corn oil (1 h pre-dose - controls), TOPT the esterase inhibitor (125 mg/kg, 18 h pre-dose) or PB, monooxygenase inhibitor (150 mg/kg, 1 h pre-dose)

All rats were observed regularly following dose administration and a comparison of PB/TOPT pre-treated animals with corn oil pre-treated control

The effects of the enzymatic inhibitors on the acute toxicity of the two test pyrethroids was investigated by using differing pre-treatment regimen and administering doses of Cypermethrin or permethrin at their respective median lethal dose.

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

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

Section A6.1.1 Acute Oral Toxicity - Rat

Annex Point IIA 6.1

Rat, neonatal and adult, two pyrethroids permethrin and Cypermethrin, acute oral toxicity

5.2 Results and discussion

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

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

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

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

Median lethal doses for permethrin:

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

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

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

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

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

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

Section A6.1.1	**Acute Oral Toxicity - Rat**
Annex Point IIA 6.1	**Rat, neonatal and adult, two pyrethroids permethrin and Cypermethrin, acute oral toxicity**

		Pre-treatment with PB or TOPT did not produce a significant variation in lethality response in Cypermethrin treated neonates (8, 16 or 21 days) but mortality was significantly increased among the adults pre-treated with the esterase inhibitor, although a similar effect was not apparent for PB. Pre-treatment of adult rats with TOPT similarly increased the percent mortality but the young rats (8, 16 or 21 days) were unaffected. Pre-treatment with PB had no effect on mortality for any of the adult or neonate rats.
5.3	**Conclusion**	The greater sensitivity of neonatal rats to pyrethroid toxicity was attributed to incomplete development of the enzymatic systems responsible for catalyzing pyrethroid metabolism. Ester hydrolysis was identified as an important detoxification pathway in the adult rat for both Cypermethrin and permethrin.
5.3.1	Reliability	2
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	January, 2011.
Materials and Methods	The applicant's version is acceptable.
Results and discussion	The applicant's version is adopted.
Conclusion	The applicant's version is adopted. Cypermethrin LD_{50} oral = 250 mg/kg bw (male adult rats)
Reliability	2
Acceptability	acceptable
Remarks	

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

Section A6.1.1 Acute Oral Toxicity - Rat

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

			Official use only
		1 REFERENCE	
1.1	**Reference**	Ravi, G.S. (2005); Acute oral toxicity study (acute toxic class method) with cypermethrin in Wistar rats; Rallis Research Centre, India, report no. 4242/05, 15 July 2005 (unpublished). Dates of work: 29th March 2005 – 22nd April 2005	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Agriphar s.a.	
1.2.2			
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I authorisation	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD guideline 423 (17th December 2001)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Cypermethrin technical (cypermethrin cis:trans/40:60)	
3.1.1	Lot/Batch number	CMN92T1197AN	
3.1.2	Specification	Certificate of Analysis supplied with study report verified the batch was within accepted specification	
3.1.2.1	Description	Viscous liquid	
3.1.2.2	Purity	94.0% w/w	
3.1.2.3	Stability	Stable – expiry date August 2006	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Wistar	
3.2.3	Source	Sri Venkateshwara Enterprises, Bangalore, India	
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	9-10 weeks, 132-152g	
3.2.6	Number of animals per group	3	
3.2.7	Control animals	No	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Post exposure period	15 days	