Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

beta-cyfluthrin (ISO); reaction mass of *rel*-(*R*)-cyano(4-fluoro-3-phenoxyphenyl)methyl (*1S,3S*)-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate and *rel*-(*R*)-cyano(4-fluoro-3-phenoxyphenyl)methyl (*1S,3R*)-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate

EC Number: -
CAS Number: 1820573-27-0

CLH-O-0000006798-55-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

4 May 2020

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name:

reaction mass of rel-(R)-cyano(4-fluoro-3-phenoxyphenyl)methyl (1S,3S)-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate and rel-(R)-cyano(4-fluoro-3-phenoxyphenyl)methyl (1S,3R)-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate (ratio 1:2);

Beta-Cyfluthrin

EC Number: **-**

CAS Number: **1820573-27-0**

Index Number: **607-254-00-7**

Contact details for dossier submitter:

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Version number: **3.0**

Date: **November 2018**

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

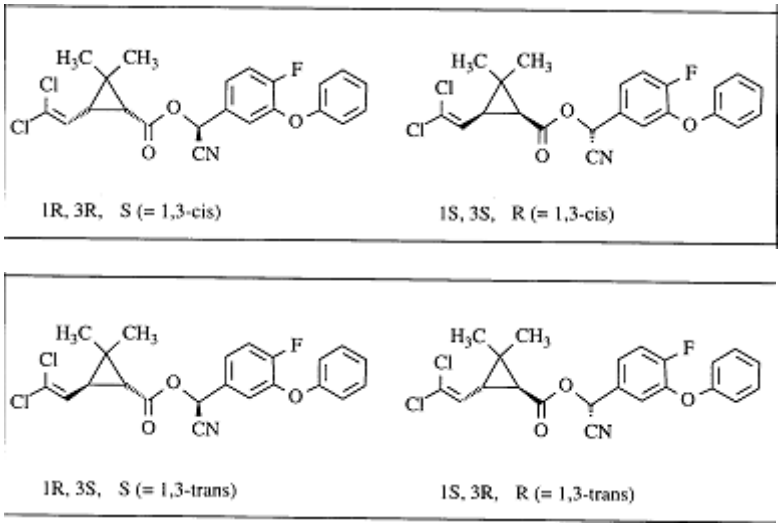
Substance name:	***Beta-Cyfluthrin***
EC number:	**-**
CAS number:	*1820573-27-0*
Annex VI Index number:	*607-254-00-7*
Degree of purity:	*≥ 96.5 %* *ratio of isomers* *diastereomer I (1R, 3R, αR + 1S, 3S, αS = 1:1, cis)* *max. 2 %* *diastereomer II (1R, 3R, αS + 1S, 3S, αR = 1:1, cis)* *30 – 40 %* *diastereomer III (1R, 3S, αR + 1S, 3R, αS = 1:1, trans)* *max. 3 %* *diastereomer IV (1R, 3S, αS + 1S, 3R, αR = 1:1, trans)* *57 – 67 %*
Impurities:	***No relevant impurities***

In the past the following identifiers were used for beta-Cyfluthrin:

	Number	Name
EC	269-855-7	α-cyano-4-fluoro-3-phenoxybenzyl 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate
CAS	68359-37-5	cyclopropanecarboxylic acid, 3-(2,2-dichloroethenyl)-2,2-dimethyl-, cyano(4-fluoro-3-phenoxyphenyl)methyl ester

Both identifiers describe a mixture of eight isomers of this structure and are also used for the substance Cyfluthrin which is actually the mixture of these eight isomers as main components.

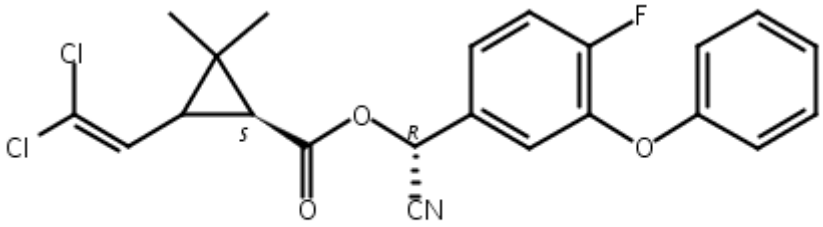
However, ß-Cyfluthrin is only a mixture of the following 4 isomers respectively two diastereomeric pairs as main components:



Due to this the EC number and CAS number used in the past are not applicable for beta-Cyfluthrin and should not be used further for the substance beta-Cyfluthrin.

The CAS No. 1820573-27-0 describes the mixture of the 4 Isomers of beta-Cyfluthrin. Therefore, this CAS No. should be used to identify beta-Cyfluthrin:

CAS Registry Number: 1820573-27-0
CAS Name: Cyclopropanecarboxylic acid, 3-(2,2-dichloroethenyl)-2,2-dimethyl-, (R)-cyano(4-fluoro-3-phenoxyphenyl)methyl ester, (1S)-rel-



relative stereochemistry

It has to be mentioned that the EC No. 269-855-7 and CAS No. 68359-37-5 are also used in the current Annex VI entry for beta-Cyfluthrin. Due to the new assigned CAS Number, and the incorrect assignment of the CAS and EC numbers in the past also the Annex VI entry should be amended accordingly.

1.2 Harmonised classification and labelling proposal

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

	CLP Regulation
Current entry in Annex VI, CLP Regulation	Acute Tox. 2*, H300 Acute Tox. 2*, H330 Aquatic Acute 1; H400 Aquatic Chronic 1; H410
Current proposal for consideration by RAC	Acute Tox. 2, H300 Acute Tox. 2, H330 Lact. H362 STOT SE 3, H335 Aquatic Acute 1; H400 Aquatic Chronic 1; H410 acute M-factor: 1 000 000 chronic M-factor: 100 000
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute Tox. 2, H300 Acute Tox. 2, H330 Lact. H362 STOT SE 3, H335 Aquatic Acute 1; H400 Aquatic Chronic 1; H410 acute M-factor: 1 000 000 chronic M-factor: 100 000

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	None		None	Conclusive but not sufficient for classification
2.2.	Flammable gases	None		None	Not applicable to solids
2.3.	Flammable aerosols	None		None	Not applicable to solids
2.4.	Oxidising gases	None		None	Not applicable to solids
2.5.	Gases under pressure	None		None	Not applicable to solids
2.6.	Flammable liquids	None		None	Not applicable to solids
2.7.	Flammable solids	None		None	Conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	None		None	Conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	None		None	Not applicable to solids
2.10.	Pyrophoric solids	None		None	Data lacking
2.11.	Self-heating substances and mixtures	None		None	Conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	None		None	Conclusive but not sufficient for classification
2.13.	Oxidising liquids	None		None	Not applicable to solids
2.14.	Oxidising solids	None		None	Conclusive but not sufficient for classification
2.15.	Organic peroxides	None		None	Data lacking
2.16.	Substance and mixtures corrosive to metals	None		None	Conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	Acute Tox. 2, H300		Acute Tox. 2*, H300	
	Acute toxicity - dermal	None		None	Conclusive but not sufficient for classification.
	Acute toxicity - inhalation	Acute Tox. 2, H330		Acute Tox. 2*, H330	
3.2.	Skin corrosion / irritation	None		None	Conclusive but not sufficient for classification.
3.3.	Serious eye damage / eye irritation	None		None	Conclusive but not sufficient for classification.
3.4.	Respiratory sensitisation	None		None	Data lacking

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
3.4.	Skin sensitisation	None		None	Conclusive but not sufficient for classification.
3.5.	Germ cell mutagenicity	None		None	Conclusive but not sufficient for classification.
3.6.	Carcinogenicity	None		None	Conclusive but not sufficient for classification.
3.7.	Reproductive toxicity	Lact. H362		None	
3.8.	Specific target organ toxicity –single exposure	STOT SE 3, H335		None	
3.9.	Specific target organ toxicity – repeated exposure	None		None	Conclusive but not sufficient for classification.
3.10.	Aspiration hazard	None		None	Data lacking
4.1.	Hazardous to the aquatic environment	Aquatic Acute 1; H400 Aquatic Chronic 1; H410	Acute M-factor: 1 000 000 Chronic M-factor: 100 000	Aquatic Acute 1; H400 Aquatic Chronic 1; H410	
5.1.	Hazardous to the ozone layer	None		None	Data lacking

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

Table 4: Proposed labelling based according to the CLP Regulation

	Labelling	Wording
Pictograms		GHS06 GHS09 (Hazardous to the aquatic environment)
Signal Word	Danger	Dgr
Hazard statements	H300 H330 H362 H335 H410	Fatal if swallowed Fatal if inhaled May cause harm to breast-fed children May cause respiratory irritation Very toxic to aquatic life with long lasting effects
Suppl. Hazard statements		

Proposed notes assigned to an entry: none

2 BACKGROUND TO THE CLH PROPOSAL

No active REACH registrations available on 9 May 2017.

2.1 History of the previous classification and labelling

Regarding health hazards, beta-cyfluthrin (CAS-No. 68359-37-5) has a legal classification (regulation (EC) No 1272/2008) for the toxicological endpoints acute oral and acute inhalation toxicity (Acute Tox. 2*, H300 Fatal if swallowed; Acute Tox. 2*, H330 Fatal if inhaled).

2.2 Short summary of the scientific justification for the CLH proposal

During the evaluation process/approval procedure of the biocidal active substance cyfluthrin in the frame of the Biocides Directive 98/8/EC and the renewal procedure for beta-cyfluthrin under Regulation (EC) No 1107/2009, it was noted that this current legal classification should be amended to include a classification for:

- reproductive toxicity (Lact. H362), based on the evidence of coarse tremors in the offspring due to cyfluthrin exposure via breast milk during lactation and
- specific target organ toxicity after single exposure (STOT-SE 3, H335), based on signs of respiratory irritation observed in humans in a volunteer study, in humans during handling of the active substance and in appropriate animal teratogenicity studies with inhalative exposure of cyfluthrin.

The increased frequency of microphthalmia observed in developmental toxicity studies with inhalation exposure was regarded to be not relevant by the dossier submitter. However, during the renewal procedure for beta-cyfluthrin under Regulation (EC) No 1107/2009 it was noted that these findings may trigger classification as Repro2 H361d.

The increased frequency of microphthalmia was discussed during the Pesticides Peer Review Meeting 172. A proposal for classification as developmental toxicant cat 2 (H361 d “Suspected of damaging the unborn child” was agreed by majority of experts. However, the dossier submitter maintains the proposed classifications as presented in Table 2.

The existing classification for the acute oral and inhalation endpoints and the non-classification regarding the remaining toxicological endpoints, other than effects on or via lactation and STOT-SE 3, is considered appropriate (see Table 3). Therefore, only the toxicological data concerning acute toxicity, irritation properties (skin, eyes, respiratory tract) as well as reproduction toxicity, relevant for the evaluation of the existing and newly proposed hazards, are reported in this CLH dossier.

Additionally, beta-cyfluthrin is currently listed in Annex VI to the CLP as acute and chronic hazardous to the aquatic environment. However, no harmonised acute/chronic M-factor is listed in Annex VI.

2.3 Current harmonised classification and labelling

Current harmonized classification and labelling regarding health hazards according to Regulation (EC) No 1272/2008 for beta-cyfluthrin (CAS-No. 68359-37-5) is *Acute Tox. 2*, H300 Fatal if swallowed* and *Acute Tox. 2*, H330 Fatal if inhaled*.

Current harmonized classification and labelling regarding environmental hazards according to regulation (EC) No 1272/2008 for beta-cyfluthrin is *Aquatic Acute 1, H400 Very toxic to aquatic life* and *Aquatic Chronic 1, H410 Very toxic to aquatic life with long lasting effects*.

2.4 Current self-classification and labelling

Table 5: C&L notifications for cyfluthrin and beta-cyfluthrin (November 2018, www.echa.eu)

Classification		Labelling			Specific Concentration limits, M-Factors
Hazard Class and Category Code(s)	Hazard Statement Code(s)	Hazard Statement Code(s)	Supplementary Hazard Statement Code(s)	Pictograms, Signal Word Code(s)	
Acute Tox. 2	H300	H300		GHS09	M=1000
Acute Tox. 3	H331	H331		GHS06	
Aquatic Acute 1	H400			Dgr	
Aquatic Chronic 1	H410	H410			
Acute Tox. 2	H300	H300		GHS09	
Acute Tox. 2	H330	H330		GHS06	
Aquatic Acute 1	H400	H400		Dgr	
Aquatic Chronic 1	H410	H410			
Acute Tox. 2	H300	H300+H330		GHS09	M(Chronic)=1000
Acute Tox. 2	H330			GHS06	M=1000
Aquatic Acute 1	H400			Dgr	
Aquatic Chronic 1	H410	H410			
Acute Tox. 2	H300	H300		GHS09	
Acute Tox. 3	H331	H331		GHS06	
Aquatic Acute 1	H400			Dgr	
Aquatic Chronic 1	H410	H410			
Acute Tox. 2	H300	H300 (H300)		GHS09	
Acute Tox. 2	H330	H331 (H331)		GHS06	
Aquatic Acute 1	H400			Dgr	
Aquatic Chronic 1	H410	H410 (H410)			
Acute Tox. 2	H300	H300		GHS09	
Acute Tox. 3	H331	H331		GHS06	
Aquatic Acute 1	H400	H400		Dgr	
Aquatic Chronic 1	H410	H410			
Acute Tox. 2	H300	H300		GHS09	
Acute Tox. 2	H330	H330		GHS06	
Aquatic Acute 1	H400			Dgr	
Aquatic Chronic 1	H410	H410			

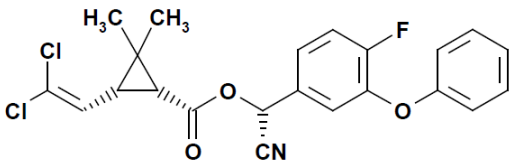
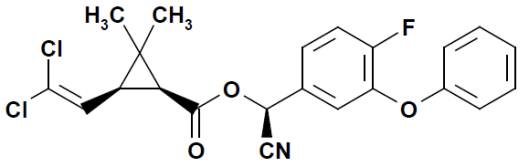
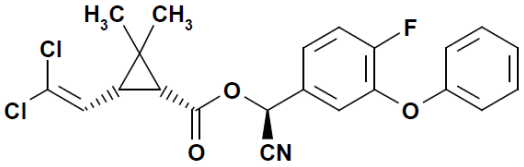
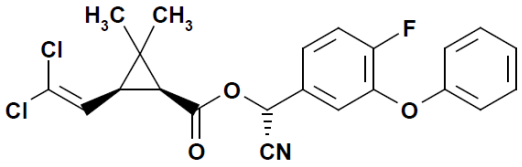
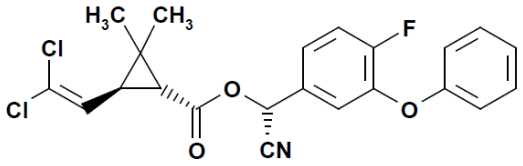
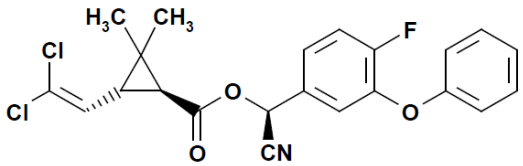
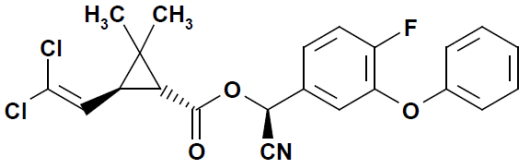
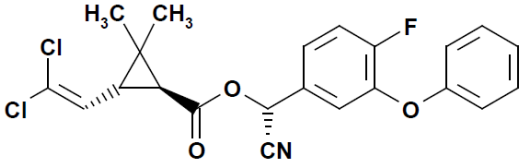
RAC general comment

Read-across for human health hazards

Cyfluthrin and beta-cyfluthrin are pyrethroid insecticides, belonging to the alpha-cyano, or Type II group of pyrethroids. Cyfluthrin is used in biocidal products and beta-cyfluthrin in plant protection products.

The dossier submitter (DS) proposed read-across between the two substances for all human health hazards evaluated and informed that read-across was generally accepted for the biocidal (cyfluthrin) and plant protection (beta-cyfluthrin) evaluation.

The molecule has 3 chiral centres, giving rise to 4 enantiomeric pairs denoted by the dossier submitter (DS) as diastereomer I to IV (see the table below). Cyfluthrin contains all four pairs in approximately equal amounts (ca. 20-35% each) while in beta-cyfluthrin pairs II and IV predominate (30-40% and 56-67% of pair II and pair IV respectively; sum of pairs I and III is below 5%).

I	 cyclopropane: 1R,3R (cis); cyano: R	 cyclopropane: 1S,3S (cis); cyano: S
II	 cyclopropane: 1R,3R (cis); cyano: S	 cyclopropane: 1S,3S (cis); cyano: R
III	 cyclopropane: 1R,3S (trans); cyano: R	 cyclopropane: 1S,3R (trans); cyano: S
IV	 cyclopropane: 1R,3S (trans); cyano: S	 cyclopropane: 1S,3R (trans); cyano: S

Biological activity (insecticidal activity and neurotoxicity to mammals) of pyrethroids significantly depends on stereochemistry. The molecule is probably active only as a whole (no molecular moiety could be identified as the toxophore) and not all stereoisomers fit equally well to the site of action (Soderlund *et al.*, 2002). Beta-cyfluthrin is a more potent insecticide than cyfluthrin.

A comparison of acute studies indicates that beta-cyfluthrin may be somewhat more potent than cyfluthrin also in mammals (see the table below).

Endpoint	**Species, experimental conditions**	**Results (reference)**	
		Cyfluthrin	**Beta-cyfluthrin**
Acute oral toxicity	Rat (Wistar), vehicle PEG 400	LD_{50} 590/1190 mg/kg bw (m/f; study 11)	LD_{50} 380/650 mg/kg bw (m/f; study 21)
	Rat (Wistar), vehicle acetone/peanut oil	LD_{50} 155/160 mg/kg bw (m/f; study 12)	LD_{50} 84/77 mg/kg bw (m/f; study 22)
	Rat (Wistar), vehicle aqueous Cremophor	LD_{50} 14-20 mg/kg bw (m; studies 1-8)	LD_{50} 11 mg/kg bw (m; Anonymous, 1986)
	Mouse, vehicle PEG 400	Strain: NMRI LD_{50} 290/610 mg/kg bw (m/f; study 14)	Strain: Bor:WISW LD_{50} 91/170 mg/kg bw (m/f; study 25)
Acute inhalation toxicity	Rat (Wistar), vehicle ethanol/PEG 400 (1:1), head-nose only	LC_{50} 0.41 mg/L (m+f; study 30)	LC_{50} 0.09/0.10 mg/L (m/f; study 35) LC_{50} 0.08 mg/L (m, f; study 36)

m=males; f=females

For repeated dose toxicity, a comparison of the available studies does not indicate a marked qualitative or quantitative difference in the toxicological profile (see for example the studies in the following table).

Endpoint	**Species, experimental conditions**	**Results (reference)**	
		Cyfluthrin	***Beta*-cyfluthrin**
Repeat dose oral toxicity	Rat, dietary, 90-d	Strain: SD Abnormal gait and salivation at 1000 ppm; no significant effects at 300 ppm (study 59)	Strain: Wistar Abnormal gait and poor general condition at 500 ppm; no effects at 125 ppm (study 62)
	Beagle dog, dietary	1-y Abnormal gait and postural reaction deficits at 360 ppm; no effects at 100 ppm (study 60)	90-d Abnormal gait at 360 ppm; no effects at 60 ppm (study 63)
Repeat dose inhalation toxicity	Rat (Wistar), 4-w, vehicle ethanol/PEG 400 (1:1)	Ruffled coat, hyperactivity and bradypnoea at 47 mg/m^3; transient bradypnoea at 6.0 mg/m^3 (Anonymous, 1989)	Piloerection, increased activity and decreased respiratory rate at 24 mg/m^3; decreased respiratory rate at 2.7 mg/m^3 (study 67)

RAC agrees to consider the data for both substances together for all human health hazards except for acute toxicity. For acute oral and inhalation toxicity, the read-across is not applied as there is conclusive data for each substance and there appears to be a certain difference in potency.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Beta-cyfluthrin is an active substance in the meaning of Regulation (EC) 1107/2009 (repealing Directive 91/414/EEC) and therefore subject to harmonised classification and labelling (Regulation EC 1272/2008).

Part B.

SCIENTIFIC EVALUATION OF THE DATA

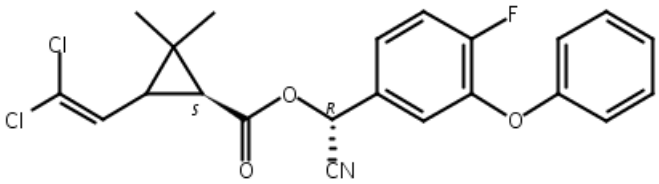
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

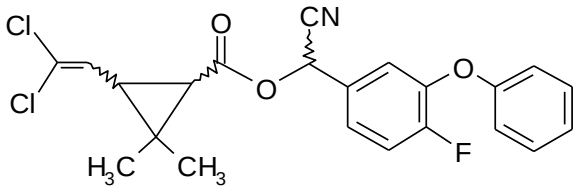
Table 6: Substance identity

EC number:	-
EC name:	-
CAS number (EC inventory):	-
CAS number:	1820573-27-0
CAS name:	Cyclopropanecarboxylic acid, 3-(2,2-dichloroethenyl)-2,2-dimethyl-, (R)-cyano(4-fluoro-3-phenoxyphenyl)methyl ester, (1S)-rel-
IUPAC name:	reaction mass of rel-(R)-cyano(4-fluoro-3-phenoxyphenyl)methyl (1S,3S)-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate and rel-(R)-cyano(4-fluoro-3-phenoxyphenyl)methyl (1S,3R)-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate (ratio 1:2)
CLP Annex VI Index number:	607-254-00-7
Molecular formula:	$C_{22}H_{18}Cl_2FNO_3$
Molecular weight range:	434.3 g/mol

Structural formula:



relative stereochemistry



1.2 Composition of the substance

Table 7: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
-	-	-	-

Table 8: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
-	-	-	-

Table 9: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
-	-	-	-	-

1.2.1 Composition of test material

The active substance as defined in the ISO common name and as reflected in the CA name is a mixture of 2 diastereomers. Each diastereomer is racemic. The minimum purity for the sum of these two diastereomers is 965 g/kg with: 300 - 400 g/kg for diastereomer II and 570 - 670 g/kg for diastereomer IV.

1.3 Physico-chemical properties

Table 10: Summary of physico - chemical properties

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
State of the substance at 20°C and 101,3 kPa	Solid	Visual	Measured
Melting/freezing point	99.4 % (beta-cyfluthrin isomer II) 78.9 °C 99.2 % (beta-cyfluthrin isomer IV) 104.3 °C	Smeykal (2012)	Measured
Boiling point	99.4 % (beta-cyfluthrin isomer II) The test item showed no boiling point at atmospheric conditions because it decomposed first starting at a temperature of 260 °C (glass crucibles) and 270 °C (aluminium crucibles). 99.2 % (beta-cyfluthrin isomer IV) The test item showed no boiling point at atmospheric conditions because it decomposed first starting at a temperature of 255 °C (glass crucibles) and 260 °C (aluminium crucibles).	Smeykal (2012)	Measured
Relative density	d_4^{22} = 1.35 at 20°C	Ziemer, Strunk (2013	Measured

Vapour pressure	99.4 % (beta-cyfluthrin isomer II) 4.5×10^{-7} Pa (20 °C) 1.0×10^{-6} Pa (25 °C) 4.5×10^{-5} Pa (50 °C) extrapolated from measurements between 73.2 °C and 111.4 °C 99.2 % (beta-cyfluthrin isomer IV) 2.2×10^{-6} Pa (20 °C) 4.6×10^{-6} Pa (25 °C) 1.2×10^{-4} Pa (50 °C) extrapolated from measurements between 79.6 °C and 129.8 °C	Smeykal (2012)	Measured
Surface tension	Not applicable, as water solubility < 1 mg/L.	Ziemer (2013)	Estimated
Water solubility	2.1 µg/L (Isomer II) and 1.6 µg/L (Isomer IV) at 20°C	Ziemer (2013)	Measured
Partition coefficient n-octanol/water	99.4 % (beta-cyfluthrin isomer II) log $P_{o/w}$ = 5.9 (25 °C; pH 5.6) 99.2 % (beta-cyfluthrin isomer IV) log $P_{o/w}$ = 5.8 (25 °C; pH 5.6)	Wiche, Peschke, Ziemer (2013)	Measured
Flash point	Not applicable (melting point > 40 °C)		Estimated
Flammability	Not flammable		Estimated
Explosive properties	Not explosive	Mix, 1995	Measured
Self-ignition temperature	No self-ignition up to 400 °C.	Mix, 1995	Measured
Oxidising properties	Not applicable. The examination of the chemical structure of beta-cyfluthrin establishes that the active substance is incapable of reacting exothermically with a combustible material.		Estimated
Granulometry	-	-	Data lacking

Stability in organic solvents and identity of relevant degradation products	99.3 % (cyfluthrin isomer II) Isomer II: acetone > 250 acetonitrile > 250 dichloromethane > 250 dimethylsulfoxide > 250 ethylacetate > 250 n-heptane 3.2 1-octanol 7.1 polyethyleneglycol 55 2-propanol 9.3 xylene > 250 all values in g/L at 20 °C 98.9 % (cyfluthrin isomer IV) Isomer IV: acetone > 250 acetonitrile 81 dichloromethane > 250 dimethylsulfoxide 204 ethylacetate > 250 n-heptane 1.2 1-octanol 2.8 polyethyleneglycol 27 2-propanol 4.3 xylene 103 all values in g/L at 20 °C	Gruener (2001)	Data lacking
Dissociation constant	Not applicable; the substance does not have acid or alkaline properties.	Krohn, 1994	Measured
Viscosity	-	-	-

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

Based on the following rationale, the DS concluded that beta-cyfluthrin should not be classified as flammable, oxidising or explosive.

Method	Results	Conclusion	Reference
EEC A.10 & EEC A.16	beta-cyfluthrin is not considered highly flammable and shows no signs of self-ignition.	Not classified as flammable.	Mix, 1995
EEC A.14	beta-cyfluthrin is not considered an explosive in accordance with EEC Method A.14.	Not classified as explosive.	Mix, 1995
EEC A.17	beta-cyfluthrin is not considered an oxidising substance.	Not classified as oxidising.	-

Comments received during public consultation

One MSCA commented that more recent tests are available in the AIR of beta-cyfluthrin.

Assessment and comparison with the classification criteria

RAC agrees with the proposal of the DS that no classification with regards to flammability, explosivity, or oxidising properties are warranted for beta-cyfluthrin, further based on the rationale below.

Flammability

The substance was not tested for flammable properties using UN test N.1. However, the negative result of study EEC A.10 is considered as conclusive for no classification; see ECHA guidance R.7.1.10.3.

Explosive

There are no chemical groups present in the chemical structure, which are associated with explosive properties, and hence, the classification procedure does not need to be applied. The negative result of study EEC A.14 is considered as supportive.

Oxidising properties

Beta-cyfluthrin contains fluorine, chlorine and oxygen atoms that are all chemically bound to carbon or hydrogen. Therefore, the classification procedure does not need to be applied.

The DS also proposed no classification by way of 'conclusive, but not sufficient for classification' for the hazard classes listed below. These are accompanied by rationale, which RAC derived from the information in the CLH dossier.

Self-reactive substances and mixtures

There are no chemical groups present in the molecule, which are associated with explosive or self-reactive properties, and hence, the classification procedure does not need to be applied.

Self-heating substances and mixtures

No classification is warranted because the substance is a solid having a melting point ≤ 160°C.

Substances and mixtures which in contact with water emit flammable gases

The classification procedure needs not to be applied because the organic substance does not contain metals or metalloids.

The DS did not specifically mention corrosivity to metals, even though this was open for PC as 'conclusive, but not sufficient for classification'. However, as the stated melting point is above 55°C, no classification is warranted.

The hazard classes listed below where opened for PC but where stated as having 'data lacking'.

Organic peroxides

RAC concludes that no classification is warranted as the substance does not fall under the definition of organic peroxides according to GHS and the relevant 'UN Manual of tests and criteria' (Seventh revised edition, 2019), and not due to 'data lacking'.

Pyrophoric solids

No data is available for beta-cyfluthrin and no read-across justification from cyfluthrin has been provided for physical properties. RAC notes that if a substance does not ignite upon contact with a very hot flame (as in an A.10 test) or upon heating, it will not ignite spontaneously at room temperature. Thus, beta-cyfluthrin does not meet the criteria for classification and no further information is needed.

RAC considers that there is sufficient information available to conclude on **no classification with regards of all physical hazards on beta-cyfluthrin**.

2 MANUFACTURE AND USES

2.1 Manufacture

2.2 Identified uses

Beta-Cyfluthrin is an active substance in plant protection products with uses as an insecticide and cyfluthrin is a biocidal active substance for Product Types 18 (Insecticide).

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 11: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
EEC A.10& EEC A.16	Beta-Cyfluthrin is not considered highly flammable and shows no signs of self-ignition.	Does not classify as being flammable.	Mix, 1995
EEC A.14	Beta-Cyfluthrin is not considered an explosive in accordance with EEC Method A.14.	Does not classify as being explosive.	Mix, 1995
EEC A.17	Beta-Cyfluthrin is not considered an oxidizing substance.	Does not classify as being oxidizing.	-

4 HUMAN HEALTH HAZARD ASSESSMENT

Beta-cyfluthrin (FCR 4545) and cyfluthrin (FCR 1272) have the same chemical structure (see figure below). The common molecular structure shows three asymmetric carbon atoms. These lead to four diastereomers, each consisting of an enantiomer pair. While cyfluthrin consists of all four diastereomers (referred to as diastereomer I, II, III and IV), beta-cyfluthrin consists of the two most active diastereomers II and IV (diastereomer II: 30.0 – 40.0 %, diastereomer IV: 57.0 – 67.0 % of the sum of the four diastereoisomers; see Table 12).

Read-across of beta-cyfluthrin and cyfluthrin properties is considered scientifically appropriate and was generally accepted for the biocidal (cyfluthrin) and plant protection evaluation (beta-cyfluthrin) due to the very similar toxicological profile of both substances. Also because beta-cyfluthrin contains the biologically most active diastereomers at about 40% also contained in cyfluthrin, the lowest dose of adverse effects for each study endpoint was taken into account. Specifically the read-across applies for systemic and/or local toxicity and all routes of exposure for both substances. Hence, it is concluded that studies with beta-cyfluthrin can be applied to cyfluthrin risk assessment, and vice versa. Consequently, the entire acceptable data set of beta-cyfluthrin and cyfluthrin is considered in this dossier.

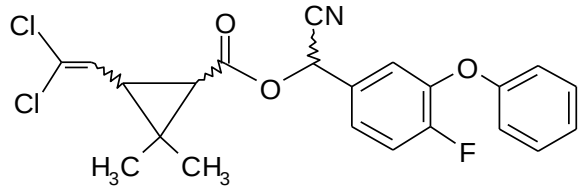


Figure 1: Structural formula of cyfluthrin

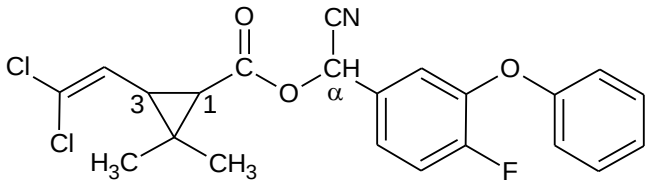


Figure 2: Diastereoisomeric pairs of beta-cyfluthrin

Table 12: Isomer compositions of cyfluthrin and beta-cyfluthrin

Diastereomer		Cyfluthrin	Beta-Cyfluthrin (FCR 4545)
I.	**1R - 3R - αR** **1S - 3S - αS**	23-27 %	≤2 %
II.	**1R - 3R - αS** **1S - 3S - αR**	17-21 %	30-40 %
III.	**1R - 3S - αR** **1S - 3R - αS**	32-36 %	≤3 %
IV.	**1R - 3S - αS** **1S - 3R - αR**	21-25 %	57-67 %

4.1 Absorption, distribution, metabolism and excretion in mammals (ADME)

No significant differences in toxicokinetic behaviour between beta-cyfluthrin and cyfluthrin were observed. Thus, the toxicokinetic data on cyfluthrin are considered representative for beta-cyfluthrin and vice versa, further supporting a read-across of toxicological data for systemic and/or local toxicity and all routes of exposure.

Study 86 investigated the metabolic fate of the cyclopropyl-moiety of the molecule ([cyclopropane-1-^{14}C] beta-cyfluthrin), using PEG 300 as a vehicle. This moiety was not investigated in the older dataset on cyfluthrin (see Table 14) and is thus considered to complete the assessment of the metabolic fate of beta-cyfluthrin.

To address an additional point in the new data requirements of Regulation 283/2013, a comparative *in vitro* metabolism study in rat/human liver microsomes has been included (study 83, Table 13). Species differences in the intrinsic clearance and the enzymes involved in the metabolism of pyrethroid pesticides were examined in rat and human hepatic microsomes. Different pyrethroids

including beta-cyfluthrin were incubated in rat and human hepatic microsomes in the presence or absence of NADPH. Metabolism was measured using a parent depletion approach. The intrinsic clearance of the majority of pyrethroids was 5 to 15-fold greater in rat relative to human microsomes. The metabolism of beta-cyfluthrin in microsomes from both species was metabolized by both oxidative and hydrolytic pathways. Rat cytochrome P450 isoforms that showed activity toward several pyrethroids included CYP1A1, CYP1A2, CYP2C6, CYP2C11, CYP3A1, and CYP3A2. Human P450 isoforms that showed activity toward multiple pyrethroids were CYP2C8, CYP2C9, CYP2C19, and CYP3A4. Species-specific differences in metabolism may result in variable detoxification of pyrethroids, which may in turn result in divergent neurotoxic outcomes. These species differences and isomer interactions in metabolism of pyrethroids should be considered when assessing the potential adverse health effects of pyrethroid pesticides. This publication supports the results in study 84, that showed that after incubation of [fluorophenyl-UL-^{14}C]-beta-cyfluthrin with active rat liver microsomes in the presence of NADPH regeneration system the test item was extensively metabolised.

Absorption:

The previously evaluated studies with cyfluthrin on rats showed a high degree of absorption (approximately 90 %: 50 % urinary, 12 % faecal, 33 % biliary, a fraction of the total amount via the bile was subject to an enterohepatic circulation) of the radioactivity. The biliary value is based on the experiments with bile cannulated animals. Unfortunately, from the three new toxicokinetic studies (study 85,86,87), information about radioactivity present in bile was not provided since the animals were not bile cannulated. Therefore, it cannot be assumed that this proportion represents material which had undergone systemic absorption. For beta-cyfluthrin a minimum absorption of 60 % can be derived from these studies (single oral low and high dose: 0.5 and 10 mg/kg bw).

The extent of absorption depends largely on the polarity of the formulation vehicle. Cyfluthrin in Cremophor EL/distilled water is absorbed faster (maximum 1 hour) and more intensively than cyfluthrin in polyethylene glycol 400 (maximum 6 hours). Accordingly, rats receiving cyfluthrin in Cremophor EL/distilled water showed signs of toxicity (i.e. hypersalivation, piloerection, diarrhea) whereas rats receiving cyfluthrin in polyethylene glycol 400 had no symptoms (study 88).

Approximately one third of the retrieved radioactivity was excreted via bile fluid during the first 2 hours and more than 90 % within the first 6 hours post application. Relating these results to the faecal excretion of intact rats following both routes of administration, it can be stated that at least one half of the faecally excreted radioactivity is due to an absorbed and biliary eliminated amount. A part of the biliary radioactivity is subject to entero-hepatic circulation (study 89).

Distribution:

The radioactivity is slowly distributed into the tissues and the distribution of radioactivity from the intravascular space into the tissues is low (study 89 and 90). The highest values were found in fatty tissue, adrenals, kidney and liver in each case. At the end of the studies (up to 10 days after administration) very low levels were found in the brain, spleen, testes, erythrocytes and plasma. Maximum relative plasma concentrations were reached 2 hours after oral administration of the low dose or the high dose. The plasma concentrations were around 1.2 times higher in the females than those measured in the males (study 85,86,87,89).

After oral administration of 10 mg/kg bw cyfluthrin, at the time of maximum plasma level (1.5 hours after administration) values in the liver and in the kidneys were markedly higher in comparison to other organs/tissues. Parallel to the onset of excretion in urine and bile, a slow redistribution of radioactivity into the fatty tissue occurs (study 90).

Metabolism:

The new studies submitted for renewal were conducted with beta-cyfluthrin. The test substance was either radiolabelled in the fluorophenyl- (study 5) or in the cyclopropyl-moiety (study 4), of the molecule.

The investigation of the metabolite pattern in urine and faeces revealed that beta-cyfluthrin was extensively metabolised independent of dose and sex.
When radiolabelled in the cyclopropyl-moiety urinary metabolite pattern consisted of at least 6 metabolite fractions.

The main metabolites in urine are a glucuronide conjugate of 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (DCVA acyl glucuronide, 26.3 - 39.1 % of recovered radioactivity) and cis/trans DCVA (25.7 - 48.8 %). All other fractions were ≤ 3 % of dose. No unchanged parent was detected in urine whereas it was the major test-related material found in faeces.

The faecal metabolite pattern revealed at least 9 metabolite fractions. The metabolite pattern was dominated by three major fractions: cis/trans DCVA accounted for 7.9 and 8.4 % in males for the high and low dose respectively, and for 4.6 and 4.7 % in females for the high and low dose, respectively. Unchanged beta-cyfluthrin was found from 14.9 - 7.7 % in males for the high and low dose, respectively, and from 26.5 - 7.6 % in females for the high and low dose, respectively. The proposed metabolic pathway is the following: beta-cyfluthrin → DCVA → DCVA glucuronide conjugate (study 4).

When radiolabelled in the fluorophenyl-moiety the main metabolites in urine after 48 h are a sulphate conjugate of OH-FPB (46.7 % of recovered radioactivity), its free form (2 %) and FPB-acid (14.6 %). Only 0.5 % unchanged parent compound was detected in the urine while the parent compound was the major test substance related material detected in faeces (20.03 %) (study 87).

In metabolism studies with cyfluthrin, 65 – 72 % of the recovered radioactivity in the dose groups A and B (both single low dose) and approximately 82 % in the dose groups C (multiple low dose groups) and D (single high dose) which were eliminated via the urine and faeces could be identified. The main metabolites were a conjugate of 4'-hydroxy-4-fluoro-3-phenoxybenzoic acid (OH-FPB acid; 51 – 52 % of recovered radioactivity), its free form ("FCR 3145", 3.0 - 5.0 % of recovered radioactivity) and 4-fluoro-3-phenoxybenzoic acid (FPB-acid, approx. 10 % of recovered radioactivity). The unchanged parent compound FCR1272 accounted for approximately half of the faecally eliminated portion (study 91).

The first step in the process of biotransformation is the cleavage of the ester bond and oxidation to FPB-acid, which then undergoes further hydroxylation and conjugation or is bound to glycine with formation of the appropriate hippuric acids. Depending upon the dose groups, unchanged parent compound and metabolites account for 65 – 82 % of the recovered radioactivity and 4 – 8 % of the radio-activity was unextractable. The metabolism is slightly dose-dependent, with the proportion of the OH-FPB acid conjugate decreasing with dose and the proportion of FPB-acid increasing with dose.

A common metabolic scheme for cyfluthrin in rats, hens and cows has been established and is depicted in Figure 3.

As demonstrated in the bile cannulation study with cyfluthrin, the parent found in faeces was absorbed and subject to enterohepatic circulation. Like with cyfluthrin, the first step in the process of biotransformation is the cleavage of the ester bond and oxidation to FPB-acid, which then undergoes further hydroxylation and conjugation. Unchanged parent compound and metabolites account for

25.46 % after 48 hours of the recovered radioactivity and 1.13 % of the radioactivity was unextractable. A metabolic scheme for beta-cyfluthrin in rats has been established and is depicted in Figure 3.

Moreover, a comparative *in vitro* metabolism study of [fluorophenyl-UL-^{14}C] beta-cyfluthrin (study 84) revealed that after adding to liver microsomes [^{14}C] beta-cyfluthrin was rapidly and more extensively metabolised in rat than in human liver microsomes. All metabolites observed with human material have also been observed in rat material. It is thus concluded that the available safety dataset in the rat is relevant and there is no unique human metabolite that would deserve further attention in risk assessment.

Elimination:

Cyfluthrin and beta-cyfluthrin are eliminated fast from the body. Thus, > 97 % of the orally and intravenously administered dose had been eliminated from the body after two days.

Beta-cyfluthrin and cyfluthrin were predominantly excreted via urine and faeces (renal/faecal: approx. 2:1). Excretion via expired gases is small, 48 hours after the oral administration of 10 mg/kg bw cyfluthrin, less than 0.001 % of the administered dose is expired (study 90). The amount of radioactivity excreted is proportional to the dose levels tested and independent of the sex of the animals.

Accumulation:

The kinetics of excretion of beta-cyfluthrin and cyfluthrin and, as well as the concentration curves in the individual tissues and organs, indicate that these substances do not accumulate, but are continuously eliminated.

Table 13: ADME studies with beta-cyfluthrin

Study Type	Test substance Dosing regime	Scope of study	Reference
Absorption, Distribution and Excretion of [fluorophenyl-UL-^{14}C] beta-cyfluthrin in Male Rats After Single Oral Administration at One Dose Level (GLP: yes; OECD TG 417)	Beta-cyfluthrin, fluorophenyl-UL-^{14}C, radiochemical purity 99.3% 10 mg/kg bw (single oral) Wistar rats , 4 males /group Vehicle: Cremophor EL	Absorption, tissue distribution, excretion pattern und kinetics. No metabolism.	study 85 †
Absorption, Distribution, Excretion and Metabolism of [fluorophenyl-UL-^{14}C] Beta-Cyfluthrin in Male Rats After Single Oral Administration at One Dose Level. (GLP: yes; OECD TG 417)	Beta-cyfluthrin, fluorophenyl-UL-^{14}C, radiochemical purity 99.3% 10 mg/kg bw (single oral) Wistar rats , 4 males /group Vehicle: PEG400	Absorption, tissue distribution, metabolism, excretion pattern und kinetics	study 87 †
Absorption, Distribution, Excretion and Metabolism of [cyclopropane-1-^{14}C] Beta-Cyfluthrin in Male and Female Rats After Single Oral Administration at Two Dose Levels. (GLP: yes; OECD TG 417)	Beta-cyfluthrin, cyclopropane-1-^{14}C, radiochemical purity 99.3% 0.5, 10 mg/kg bw (single oral) Wistar rats , 4 males and 4 females /group Vehicle: PEG400	Absorption, tissue distribution, metabolism, excretion pattern und kinetics	study 86 †
Comparative *in vitro* Metabolism of [fluoro-phenyl-UL-^{14}C] beta-cyfluthrin in Rat and Human Liver Microsomes. (GLP: yes, Guideline: no)	Beta-cyfluthrin, fluorophenyl-UL-^{14}C, radiochemical purity 99.3% 10 µM	*In vitro* comparison of metabolism in rat and human liver microsomes	study 84 †
In vitro metabolism of pyrethroid pesticides by rat and human hepatic microsomes and cytochrome P450 isoforms (GLP and guideline not applicable)	Beta-cyfluthrin and other pyrethroid pesticides purity >98%, different vehicles	In vitro metabolism in rat and human microsomes	study 83

†Key study

Table 14: ADME studies with cyfluthrin

Study Type	Test substance Dosing regime	Scope of study	Reference
Comparative study of rats on absorption of FCR 1272 after single oral administration in polyethylene glycol 400 or Cremophor EL/water as formulation vehicle. (GLP: no; guideline: no, supplemental)	Cyfluthrin, isomer ratio: I 26.6%; II 19.1%; III 33.7%; IV 20.6%; purity not reported. 10 mg/kg bw (one single oral dose, ♂ only) Different Vehicles: Polyethylene glycol 400 and Cremophor EL/distilled water	Provides comparative data on oral uptake from different vehicles (PEG400 and Cremophor/water): The higher toxicity of cyfluthrin in Cremophor EL/distilled water is caused by faster and higher absorption.	study 88
Fluorophenyl-UL-14C cyfluthrin (FCR 1272) biokinetic study in rats. (GLP: no; guideline, partly OECD TG 417)	Cyfluthrin, cis/trans ratio of 42/58, purity: 97.5% a) 0.5 mg/kg bw (single i.v. or intraduodenal, ♂), b) 0.5 mg/kg bw (single oral, ♂), c) 10 mg/kg bw (single oral, ♂) d) 0.5 mg/kg bw (single oral, ♀)	Information on accumulation, absorption, excretion, and distribution over 10 days	study 90 †
Biokinetic part of the general metabolism studies in the rat. (GLP: no; guideline according to EPA specifications compatible to Directive 87/302/EEC, Part B)	Cyfluthrin, cis/trans ratio of 42/58, purity: 97.5% Vehicle: Cremophor/saline Administration (♂ only): a) 0.5 mg/kg bw (single i.v. or intraduodenal) b) 0.5 mg/kg bw (single oral) c) 0.5 mg/kg bw/day (oral: 14 nonradioactive doses + single radioactive dose) d) 10 mg/kg bw (single oral) Rat, Mura: SPRA (SPF 68 Han) *Intraduodenal/bile cannulated:* 5 males *Single oral low dose group:* 9 males + 9 females *other groups:* 5 males + 5 females	Provides mass balance and distribution of radiolabel in excreta and carcass following different routes of administration.	study 89 †
[Fluorobenzene-UL-^{14}C]cyfluthrin: Metabolism part of the general metabolism studies in the rat. (GLP: no; guideline according to EPA specifications compatible	Cyfluthrin, cis/trans ratio of 42/58, radiochemical purity: 98% Vehicle: Cremophor/saline Administration (♂ only): a) 0.5 mg/kg bw (single i.v.) b) 0.5 mg/kg bw (single oral)	Identification of metabolites in excreta	study 91 †

Study Type	Test substance Dosing regime	Scope of study	Reference
to Directive 87/302/EEC, Part B)	c) 0.5 mg/kg bw/day (oral: 14 nonradioactive doses + single radioactive dose) d) 10 mg/kg bw (single oral) Rat, Sprague Dawley (4 males and 4 females)		
Thiocyanate excretion in rats' urine after intraperitoneal administration of FCR 1272 and decamethrin in comparable doses and after exposure to defined FCR 1272 concentrations in the inhalation air. (GLP: no, Guideline: no, supplemental)	Cyfluthrin, isomer ratio: I 24.9%; II 17.9%; III 30.0%; IV 22.2%; purity: 95%; Decamethrin purity: 99.2% 0, 1, 5, 10, 15 mg/kg bw i.p. (♂ only); 0, 59, 93, 180 mg/m³ exposure via inhalation (♂+♀)	Focus on thiocyanate excretion in urine following i.p. and exposure via inhalation of cyfluthrin and decamethrin	study 92
Biotransformation of [F-phenyl-UL-14C]cyfluthrin; characterisation and preliminary identification of the metabolites. (GLP: no, Guideline: no)	Cyfluthrin, cis/trans ratio of 42/58, radiochemical purity: 98% 10 mg/kg bw oral (only ♂); vehicle not reported	Preliminary study for identification of urinary metabolites	study 93

†Key study

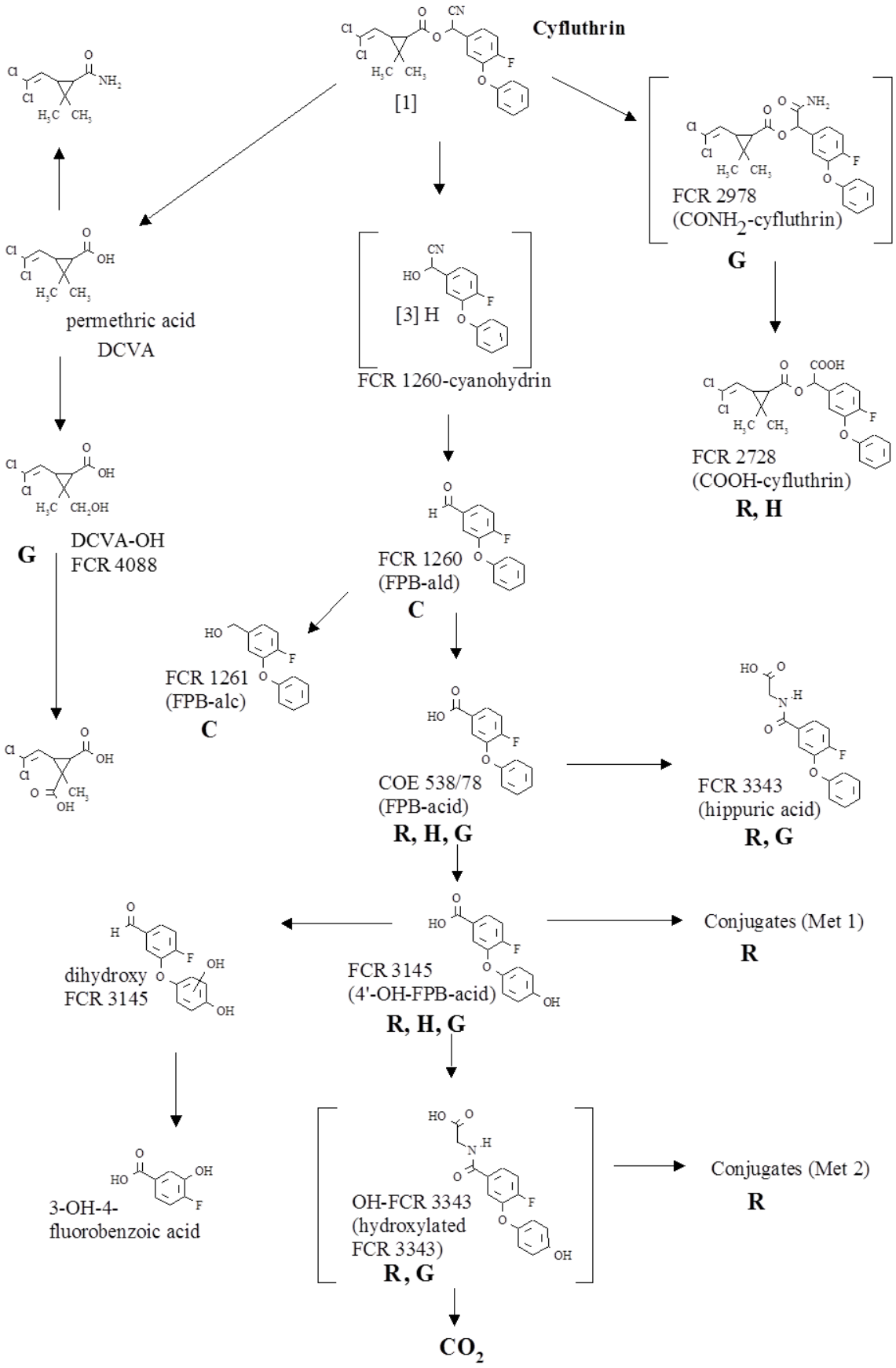


Figure 3: Proposed metabolic pathway for cyfluthrin in rats (R) , laying hens (H), cows (C) and goats (G)

Table 15: Toxicokinetics and metabolism in rats - Excretion of total radioactivity and radioactive residues in the rat 48 hours after application of [fluorophenyl-UL-14C] cyfluthrin (values are given in % of recovered radioactivity)

Report	Administration	Dose [mg/kg bw]	Sex	CO_2	Bile	Urine	Faeces	Total excreted	Ratio Urine/ Faeces	Body without GIT	GIT	Recovery (% of applied)
study 89	intraduodenal	0.5	m	-	33	54	12	99	4.5	0.5	0.15	103
	oral[1)]	10	m	<0.001	-	67	31	98	2.2	1.3	0.27	106
	intravenous	0.5	m	-	-	69	24	93	2.9	5.6	0.74	94
	oral	0.5	m	-	-	74	25	99	3.0	1.1	0.22	93
	pretreat. oral	0.5	m	-	-	73	26	99	2.8	1.2	0.24	91
	oral	10	m	-	-	66	33	99	2.0	1.4	0.23	99
	oral	10	f	<0.001	-	67	31	98	2.2	2.1	0.42	98
	intravenous	0.5	f	-	-	65	28	93	2.3	6.5	0.78	93
	oral	0.5	f	-	-	61	37	98	1.6	1.6	0.59	101
	pretreat. oral	0.5	f	-	-	63	36	99	1.8	1.2	0.32	96
	oral	10	f	-	-	52	45	97	1.2	1.6	0.45	101
study 90	intraduodenal	0.5	m	-	33.5	54.2	11.6	99.3	4.7	0.5	0.15	103.1
	oral[1)]	10	m	<0.001	-	59.1	39.3	98.4	1.5	1.4	0.30	95.0
	intravenous	0.5	m	-	-	69.5	24.1	93.6	2.9	5.7	0.75	93.5
	oral	0.5	m	-	-	74.2	24.5	98.7	3.0	1.1	0.21	93.8
	oral	0.5	w	-	-	61.7	36.7	97.4	1.7	1.6	0.60	99.3
	oral	10	m	-	-	65.9	32.4	98.3	2.0	1.4	0.25	99.4
study 91	intravenous	0.5	m	-	-	67.0	26.6	93.6	2.5	6.4		90.0
	intravenous	0.5	f	-	-	65.2	25.3	90.5	2.6	9.5		87.6
	oral	0.5	m	-	-	73.0	25.7	98.7	2.8	1.3		97.1
	oral	0.5	fw	-	-	61.4	36.5	97.9	1.7	2.1		94.0
	pretreat. oral	0.5	m	-	-	71.8	26.7	98.5	2.7	1.5		87.4
	pretreat. oral	0.5	f	-	-	62.2	35.4	97.6	1.8	2.4		93.6
	oral	10	m	-	-	65.0	33.4	98.4	1.9	1.6		94.8
	oral	10	f	-	-	59.6	37.8	97.4	1.6	2.6		96.9

GIT: gastrointestinal tract;

[1)] Preliminary study to assess the volatility of cyfluthrin.

Table 16: Toxicokinetics and metabolism in rats - Relative concentration of radioactivity (P) in individual parts of the body of rats after application of [fluorophenyl-UL-14C] cyfluthrin (all values are multiplied with the factor 100)

Report	Admini-stration	Dose (mg/kg bw)	Sex	Time (h)	Body without GIT	Plas-ma	Ery-thro-cytes	Testes or Ovaries	Femur	Brain	Skin	Heart	Spleen	Liver	Kidney	Renal fat	Adre-nal
study 7	intra-venous	0.5	m	48	6	17	4,5	1,2	2.0	0.6	6.2	3.4	13	14	5.4	53	16
	oral	0,5	m	48	1.1	0.94	0.2	0.16	0.38	0.065	1.3	0.26	0.54	2.0	1.1	16	1.4
	pretreat.oral	0.5	m	48	1.3	1.1	0.31	0.18	0.23	0.057	1.8	0.27	0.36	2.1	1.3	9	2.3
	oral	10	m	48	1.6	0.86	0.44	0.21	0.42	0.07	1.8	0.29	0.27	2.5	1.3	18	1.6
	intra-venous	0.5	f	48	6.6	18	4.7	2.7	2.8	0.57	9.7	3.9	16	15	7.4	33	24
	oral	0.5	f	48	1.8	3.2	0.56	3.2	0.54	0.13	2.2	0.67	0.48	3.4	3.2	12	3.9
	pretreat.oral	0.5	f	48	1.3	2.4	0.47	1.6	0.39	0.077	1.8	0.51	0.24	2.3	2.0	5.3	1.5
	oral	10	f	48	1.8	2.6	0.52	3.0	0.43	0.12	2.5	0.8	0.36	3.0	2.7	11	2.4
study 8	oral	10	m	1.5	44	220	48	16	15	-	35	-	22	170	130	36	73
	oral	10	m	4	33	130	30	16	10	-	29	-	14	100	85	60	32
	oral	10	m	8	21	65	12	11	5.5	-	18	-	5.8	51	46	42	10
	oral	10	m	24	4.7	12	2.6	2.2	1.6	-	5.0	-	1.6	8.3	7.0	24	5.3
	oral	10	m	48	2.0	1.6	0.51	0.35	0.72	-	1.9	-	0.61	2.8	1.5	22	10
	oral	10	m	72	1.1	0.49	0.18	0.1	0.52	-	1.1	-	0.14	1.8	0.7	17	0.89
	oral	10	m	144	0.5	0.24	0.064	0.061	0.39	-	0.29	-	0.059	0.9	~0.35	8.4	0.79
	oral	10	m	240	0.26	0.061	0.037	0.017	0.14	-	~0.13	-	0.016	~0.43	0.13	6.1	~0.19

P= measured activity/g tissue or plasma administered activity/g bw

Table 17: Toxicokinetics and metabolism in rats - Distribution of metabolites in the excreta of rats 48 hours after administration of [fluorophenyl-UL-14C]cyfluthrin. For codes of the metabolites see Figure 3 (values are given in % of the recovered radioactivity)

Report	Administra-tion	Dose (mg/kg)	Excretion	Sex	Met.1 ❶	FCR 3145	Met. 2 ❷	FCR 3343	COE 538/78	FCR 1272	Un-known	Not ex-tractable	Total
study 9	intravenous	0.5	Urine	m	47.0	2.9	1.5	2.4	12.1	-	1.1	-	67.0
	intravenous	0.5	Faeces	m	0.1	1.9	0.1	-	-	0.4	24.1	8.0	26.6
			∑		47.1	4.8	1.6	2.4	12.1	0.4	25.2	8.0	93.6
	intravenous	0.5	Urin	f	44.4	4.4	1.5	2.3	10.8	-	1.8	-	65.2
	intravenous	0.5	Faeces	f	0.2	4.9	-	-	0.3	0.5	12.1	7.3	25.3
			∑		44.6	9.3	1.5	2.3	11.1	0.5	13.9	7.3	90.5
	oral	0.5	Urine	m	52.0	3.8	2.1	3.6	10.1	-	1.4	-	73.0
	oral	0.5	Faeces	m	-	1.1	0.1	-	-	0.1	19.5	4.9	25.7
			∑		52.0	4.9	2.2	3.6	10.1	0.1	20.9	4.9	98.7
	oral	0.5	Urine	f	41.1	3.9	2.6	2.4	9.9	-	1.5	-	61.4
	oral	0.5	Faeces	f	-	4.6	0.4	0.2	0.3	0.1	23.9	7.0	36.5
			∑		41.1	8.5	3.0	2.6	10.2	0.1	25.4	7.0	97.9
	pretr.oral	0.5	Urine	m	47.4	3.2	3.0	6.7	10.5	-	1.0	-	71.8
	pretr.oral	0.5	Faeces	m	-	0.8	0.1	-	0.1	11.6	8.9	5.2	26.7
			∑		47.4	4.0	3.1	6.7	10.6	11.6	9.9	5.2	98.5
	pretr.oral	0.5	Urine	f	41.8	4.4	2.9	2.7	8.3	-	2.1	-	62.2
	pretr.oral	0.5	Faeces	f	-	6.4	-	0.3	-	16.2	8.9	3.6	35.4
			∑		41.8	11.0	2.9	3.0	8.3	16.2	11.0	3.6	97.6
	oral	10	Urine	m	35.9	1.8	0.8	0.5	24.1	-	1.9	-	65.0
	oral	10	Faeces	m	-	1.2	-	0.4	-	16.6	10.2	5.0	33.4
			∑		35.9	3.0	0.8	0.9	24.1	16.6	12.1	5.0	98.4
	oral	10	Urine	f	35.2	4.5	2.1	17.3		-	0.5	-	59.6
	oral	10	Faeces	f	-	4.3	-	-		19.0	9.5	5.0	37.8
			∑		35.2	8.8	2.1	17.3		19.0	10.0	5.0	97.4

❶: Conjugate of FCR 3145
❷: Probably conjugate of hydroxylated FCR 3343.

4.2 Acute toxicity

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

The experimental oral LD_{50} values of beta-cyfluthrin and cyfluthrin are covering a broad range. This finding could be evoked by different factors:

The acute oral toxicity of beta-cyfluthrin and cyfluthrin seems to be dependent on the vehicle used (see Table 18 and Table 19). This may be due to different polarity leading to modified absorption in the gastrointestinal tract. Furthermore, beta-cyfluthrin generally possesses, vehicle-dependently, a higher acute oral toxicity than cyfluthrin. This observation could be inferred from its particular isomer composition (beta-cyfluthrin: a large amount of more toxic isomer 2 and only a small amount of less toxic isomer 3). The lowest LD_{50} values determined in acceptable studies with beta-cyfluthrin were 77 mg/kg bw (acetone/peanut oil; study 22) in rats and 91 mg/kg bw (PEG 400; study 25) in mice. The lowest LD_{50} values determined in acceptable studies with cyfluthrin were 14.3 mg/kg bw (Cremophor/water; study 5) in rats and 291 mg/kg bw (PEG 400; study 14) in mice. As laid down in the actual CLP regulation, […] "generally the lowest valid value would be the basis for classification […] if there are different LD_{50} values from tests using different vehicles" (page 265). For this reason and taking further the read-across approach into account, the classification for acute oral toxicity for beta-cyfluthrin was based on cyfluthrin (solvent: Cremophor/water; study 5).

Table 18: Summary table of relevant acute oral toxicity studies with beta-cyfluthrin*

Parameter	Species	Sex	Vehicle	Result	Reference
acute oral LD_{50} (GLP: yes; OECD 401)	Rat (Wistar)	male female male female (5 male and 5 female/group)	PEG 400 (beta-cyfluthrin batch no.: 16002/84, purity: 99.1 %)	655 mg/kg bw[1,2] 1369 mg/kg bw[1,2] 380 mg/kg bw 651 mg/kg bw	study 21
acute oral LD_{50} (GLP: yes; OECD 401)	Rat (Wistar)	male female male female (5 male and 5 female/group)	acetone/ peanut oil (beta-cyfluthrin batch no.: 16002/84, purity: 99.1 %)	141 mg/kg bw[1,2] 108 mg/kg bw[1,2] 84 mg/kg bw 77 mg/kg bw	Study 22
acute oral LD_{50} (GLP: yes; OECD 401)	Rat (Wistar)	male female male female (5 male and 5 female/group)	xylene (beta-cyfluthrin batch no.: 16002/84, purity: 99.1 %)	307 mg/kg bw[1,2] 343 mg/kg bw[1,2] 211 mg/kg bw 336 mg/kg bw	Study 23
acute oral LD_{50} (GLP: yes; OECD 423)	Rat (Wistar)	female (3 /group)	acetone/corn oil (beta-cyfluthrin batch no.: FFEBCTQ043, purity: 99.2 %)	200 mg/kg bw	Study 24
acute oral LD_{50} (GLP: yes; OECD 401)	Mice (Bor:WISW (SPF-Han)	male female (5 male and 5 female/group)	PEG 400 Beta-cyfluthrin (batch no.: 16002/84, purity: 99.1 %)	91 mg/kg bw 165 mg/kg bw	Study 25 †

acute oral LD_{50} (GLP: no, unpublished)	Chicken(White Leghorn Hens)	5 female	cremophor/water (beta-cyfluthrin (batch no.: 16002/84, purity: 99.1 %)	>5000 mg/kg bw[1,2,3]	Study 26

* Not-acceptable studies were not included.
[1] Animals not fasted.
[2] These studies are considered supplementary.
[3] These studies were not submitted by the applicant (but available to RMS e.g. from literature search in database or other applications).
†Key study

Table 19: Summary table of relevant acute oral toxicity studies with cyfluthrin*

Parameter	Species	Sex	Vehicle	Result	Comment	Reference
acute oral LD_{50} (GLP: no, similar to OECD 401)	Rat (Wistar)	male male male male (5-20/ group)	cremophor/water acetone/oil dimethylsuphoxide *N*-methylpyrrolidone (cyfluthrin batch no. 816170019, purity 95%)	16.2 mg/kg bw 254 mg/kg bw 396 mg/kg bw 500-1000 mg/kg bw	(fasted) -preliminary LD_{50} determination -no detailed information given (e.g. doses, group size)	study 1
acute oral LD_{50} (GLP: no, similar to OECD 401)	Rat (Wistar)	5 male/ group	cremophor/water (cyfluthrin batch no.: 816270011, purity: 93.7 %)	20 mg/kg bw	-combination study -LD_{50} (cyfluthrin + propoxur) = 57 mg/kg bw -no necropsy	study 2
acute oral LD_{50} (GLP: no, similar to OECD 401)	Rat (Wistar)	5 male/ group	cremophor/water (cyfluthrin batch no.: 816270011, purity: 93.7 %)	20 mg/kg bw	-combination study -LD_{50} (cyfluthrin + dichlorvos) = 70 mg/kg bw -no necropsy	study 3
acute oral LD_{50} (GLP: no, similar to OECD 401)	Rat (Wistar)	5 male/ group	cremophor/water (cyfluthrin batch no. 816270011, purity 93.7%)	20 mg/kg bw[3]	-combination study -LD_{50} (cyfluthrin + fenfluthrin) = 67 mg/kg bw -no necropsy	study 4
acute oral LD_{50} (GLP: no, similar to OECD 401)	Rat (Wistar)	10 male	cremophor/water (cyfluthrin batch no. 816170019, purity 95%)	14.3 mg/kg bw	(fasted)	study 5 †
acute oral LD_{50} (GLP: no, similar to OECD 401)	Rat (Wistar)	5-10 male/ group	cremophor/water (cyfluthrin batch no.: 816170019, purity: 94.9 %)	18 mg/kg bw	-combination study -LD_{50} (cyfluthrin + methamidphos) = 26 mg/kg bw	study 6
acute oral	Rat	5 male/	cremophor/water	15 mg/kg bw[2]	-combination	study 7

Parameter	Species	Sex	Vehicle	Result	Comment	Reference
LD_{50} (GLP: yes, similar to OECD 401)	(Wistar)	group	(cyfluthrin batch no.: 238005176, purity: 95.1 %)		study -only two doses tested -LD_{50} (cyfluthrin + imidacloprid) = 414 mg/kg bw	
acute oral LD_{50} (GLP: no, similar to OECD 401)	rat (Wistar)	5-20 male/group	cremophor/water (cyfluthrin batch no.: 816170019, 816270030, purity: 94.9 %, 94.7 %)	19.6 mg/kg bw	-study for antidote effect -no necropsy	study 8
acute oral LD_{50} (GLP: no, similar to OECD 401)	Rat (Wistar)	5 male/ group	PEG 400 (cyfluthrin batch no.: 233690489, purity: 95.7 %)	500 mg/kg bw	-combination study -LD_{50} (cyfluthrin + omethoate) = 218 mg/kg bw	study 9
acute oral LD_{50} (GLP: no, unpublished)	Rat (Wistar)	15 male 15 female	PEG 400 (cyfluthrin batch no. 16001/79, purity: 83.6%)	869 mg/kg bw[1,2,3] 1271 mg/kg bw[1,2,3]	(animals not fasted)	study 10
acute oral LD_{50} (GLP: no, unpublished)	Rat (Wistar)	15 male 15 female	PEG 400 (cyfluthrin batch no. 16001/79, purity: 83.6%)	590 mg/kg bw[3] 1189 mg/kg bw[3]	(fasted)	study 11
acute oral LD_{50} (GLP: yes, OECD 401)	Rat (Wistar)	5-10 male and 5-10 female/ group	acetone/peanut oil (cyfluthrin batch no. 23490583, purity: 93%)	155 mg/kg bw[3] 160 mg/kg bw[3]	(fasted)	study 12
acute oral LD_{50} (GLP: no, similar to OECD 401)	Mouse (NMRI)	female	cremophor/water (cyfluthrin batch no. 816170019, purity 95%)	<100 mg/kg bw[2,3]	-preliminary LD_{50} determination -no detailed information given (e.g. doses, group size)	study 13
acute oral LD_{50} (GLP: no, unpublished)	Mouse (NMRI)	15 male 15 female	PEG 400 (cyfluthrin batch no. 16001/79, purity: 83.6%)	291 mg/kg bw[3] 609 mg/kg bw[3]		study 14 †
acute oral LD_{50} (GLP: no, unpublished)	Rabbit (White New Zeland)	3 male	PEG 400 (cyfluthrin batch no. 16001/79, purity: 83.6%)	>1000 mg/kg bw[2,3]	-only three animals per dose -no necropsy	study 15
acute oral LD_{50} (GLP: no, unpublished)	Dog (Beagle)	2 male	PEG 400 (cyfluthrin batch no. 16001/79, purity: 83.6%)	>100 mg/kg bw[2,3]	-vomiting at 50 mg/kg bw and above -only two	study 16

Parameter	Species	Sex	Vehicle	Result	Comment	Reference
					animals per dose -no necropsy	
acute oral LD_{50} (GLP: no, unpublished)	Dog (Beagle)	1 male 1 female	cremophor/water (cyfluthrin batch no. 816170019, purity 95%)	>100 mg/kg bw[1,2,3]	-vomiting observed -animals not fasted -only two animals per dose (1 per sex) -only two doses -no necropsy	study 17
acute oral LD_{50} (GLP: yes, OECD 401)	Chicken (White Leghorn Hens)	5 female/ group	cremophor/water (cyfluthrin batch no. 233590478, purity 93.5%)	>5000 mg/kg bw[1,2,3]	-animals not fasted -only two doses tested	study 18
acute oral LD_{50} (GLP: no, similar OECD 418 and 419)	Chicken (White Leghorn Hens)	10 female/ group	PEG 400 (cyfluthrin batch no.: 16001/79, purity: 85.3 %)	~5000 mg/kg bw[1,2]	-animals not fasted	study 19
acute oral LD_{50} (GLP: yes, OECD 401)	Chicken (White Leghorn Hens)	5 female/ group	PEG 400 (cyfluthrin batch no.: 233590478, purity: 93.5 %)	~4500 mg/kg bw[1,2,3]	-animals not fasted -only two doses tested	study 20

[*] Not-acceptable studies were not included.
[1] Animals not fasted.
[2] These studies are considered supplementary.
[3] These studies were not submitted by the applicant (but available to the RMS e.g. from other procedures).
†Key study

4.2.1.2 Acute toxicity: inhalation

The LC_{50} values of beta-cyfluthrin and cyfluthrin were determined in rodents after exposure to either dust or mist aerosol (see

Table 20 and

Table 21).

Based on the worst-case LC_{50} value determined in an acceptable inhalation study, the LC_{50} value in rats used for classification was 0.081 mg/L air (81 mg beta-cyfluthrin in ethanol/PEG400 /m^3 air as mist, 4h-exposure, head-nose only; study 36). The lowest rat LC_{50} value after dust exposure was 0.532 mg/L air (532 mg beta-cyfluthrin/m^3 air as dust, 4h-exposure, head-nose only; study 36). It is mentioned that the terms “dust” and “mist” and “aerosol” used by the authors of the acute inhalation studies all refer to the hazard category “dust and mists”.

Table 20: Summary table of relevant acute inhalation toxicity studies with beta-cyfluthrin*

Parameter	Species	Sex	Vehicle	Result	Reference
acute inhal. LC_{50} (4 h, head-nose) (GLP: yes OECD 403)	Rat (Wistar)	5 male + 5 female (group)	ethanol/PEG 400 (mist aerosol) beta-cyfluthrin (batch no: 16002/84, purity: 98.5 %)	~90 /~ 100 mg/m³ air (male/female) ~967 /~ 695 mg/m³ air (male/female)	Study 35
acute inhal. LC_{50} (4 h, head-nose) (GLP: yes OECD 403)	Rat (Wistar)	5 male + 5 female (group)	ethanol/PEG 400 (mist aerosol) beta-cyfluthrin (batch no: 16001/87, purity: 97.9 %)	~82 /81 mg/m³ air (male/female) 532 mg/m³ air (male + female)	Study 36 †

* Not-acceptable studies were not included.

†Key study

Table 21: Summary table of relevant acute inhalation toxicity studies with cyfluthrin*

Parameter	Species	Sex	Vehicle	Result	Comment	Reference
acute inhal. LC_{50} (1 h, nose only) (GLP: no, unpublished)	Rat (Wistar)	10 male +10 female (group)	ethanol/PEG 400 (1:1) (mist aerosol) (cyfluthrin batch no. 16001/79, purity: 83.6%)	>1089 mg/m³ air³ (male + female)	-inhalation particle content not given -no vehicle control	Study 27
acute inhal. LC_{50} (4 h, nose only) (GLP: no, unpublished)	Rat (Wistar)	10 male +10 female (group)	ethanol/PEG 400 (1:1) (mist aerosol) (cyfluthrin batch no. 16001/79, purity: 83.6%)	469-592 mg/m³ air³ (male + female)	-inhalation particle content not given -no vehicle control	Study 28
acute inhal. LC_{50} (4 h, head/nose only assumed) (GLP: no, unpublished)	Rat (Crj: CD)	male + female	ethanol/PEG 400 ethanol/PEG 400 (1:1) (mist aerosol) (cyfluthrin lot no. Eg 3/81, purity 95%)	1010 /1020 mg/m³ air³ (male/female)	-inhalation particle content not given - number of animals used not indicated.	Study 29
acute inhal. LC_{50} (4 h, head/nose only) (GLP: yes, OECD 403)	Rat (Wistar)	5 male + 5 female (group)	ethanol/PEG 400 (1:1) (mist aerosol) (cyfluthrin batch no. 233490583, purity: 93%)	405 mg/m³ air³ (male/female)	-no vehicle control	Study 30
acute inhal. LC_{50} (4 h, head/nose only) (GLP: no, unpublished)	Rat (Wistar)	male + female male + female (10 male and 10 females / group)	1) water 2) DMSO (mist aerosol) (cyfluthrin batch no.816170019, purity: 95%)	1) >735 /200-735 m³ air³ (male/female) 2) 575 /490 mg/m³ air³ (male/female)	-inhalation particle content not given -no vehicle control	Study 31
acute inhal. LC_{50} (5 x 6 h,	Rat (Wistar)	10 male +10 female	ethanol/PEG 400 (1:1) (mist	47-196 mg/m³ air[2,3] (range for	-inhalation particle	Study 32

nose only) (GLP: no, unpublished)		(group)	aerosol) (cyfluthrin batch no. 16001/79, purity: 83.6%)	male/female)	content not given -no vehicle control -no different time points	
acute inhal. LC_{50} (4 h, head/nose only) (GLP: yes, OECD 403)	Mouse (NMRI)	5 male + 5 female (group)	ethanol/PEG 400 (1:1) (mist aerosol) (cyfluthrin batch no. 233782017, purity: 93.9%)	~141 mg/m³ air[3] (male/female)		Study 33
acute inhal. LC_{50} (4 h, whole body) (GLP: no, similar to OECD 403 and 412)	Chicken (White Leghorn Hens)	10 female/group	ethanol/PEG 400 or water/cremophor (mist aerosol) (cyfluthrin batch number: 816 170 019; purity 95.0 %)	>596 mg/m³ air[2]	-inhalation particle content not given -different solvents -no vehicle control	Study 34

[*] Not-acceptable studies were not included.
[1] Animals not fasted.
[2] These studies are considered supplementary.
[3] These studies were not submitted by the applicant (but available to the RMS e.g. from other procedures).

4.2.1.3 Acute toxicity: dermal

The dermal toxicity of beta-cyfluthrin and cyfluthrin is very low (see Table 22 and

Table 23). The lowest dermal LD_{50} value in rats determined in an acceptable study with beta-cyfluthrin was used for non-classification decision (>2000 mg/kg bw, solvent: PEG 400; study 24).

Table 22: Summary table of relevant acute dermal toxicity studies with beta-cyfluthrin*

Parameter	**Species**	**Sex**	**Vehicle**	**Result**	**Reference**
acute dermal LD_{50} (GLP: yes OECD 402)	Rat (Wistar)	male + female (5 males and 5 females)	PEG 400 (eta-cyfluthrin batch no.: 16002/84, purity: 99.1 %)	>5000 mg/kg bw	Study 40
acute dermal LD_{50} (GLP: yes OECD 402)	Rat (Wistar)	male + female (5 males and 5 females)	Xylene (beta-cyfluthrin batch no.: 16002/84, purity: 99.1 %)	>5000 mg/kg bw	Study 41
acute dermal LD_{50} (GLP: yes OECD 402)	Rat (Wistar)	male + female (5 males and 5 females)	PEG 400 (beta-cyfluthrin batch no.: FFEBCTQ043, purity: 99.2 %)	>2000 mg/kg bw	Study 42 †

* Not-acceptable studies were not included.
†Key study

Table 23: Summary table of relevant acute dermal toxicity studies with cyfluthrin*

Parameter	Species	Sex	Vehicle	Result	Comment	Reference
acute dermal LD_{50} (GLP: no, similar to OECD 401)	Rat (Wistar) (24 h contact)	male female (5-10 male and 5-10 female)	cremophor/water (cyfluthrin batch no.: 816170019, 816270030, purity: 94.9 %, 94.7 %)	>5000 mg/kg bw[2,3]	-only two doses tested (limit test not sufficient) -unclear which sex was used at lower concentration -no necropsy	Study 37
acute dermal LD_{50} (GLP: no, similar to OECD 401)	Rat (Wistar) (24 h contact)	male female (5-10 male and 5-10 female)	PEG 400 (cyfluthrin batch no.: 816170019, 816270030, purity: 94.9 %, 94.7 %)	>5000 mg/kg bw[2,3]	-no neropsy -no detailed information given	Study 38
acute dermal LD_{50} (GLP: no, similar to OECD 401)	Rat (Wistar) (24 h contact)	male female (5-10 male and 5-10 female)	NaCl solution (cyfluthrin batch no.: 816170019, 816270030, purity: 94.9 %, 94.7 %)	>5000 mg/kg bw[2,3]	-only two doses tested (limit test not sufficient) -unclear which sex was used at lower concentration -no necropsy	Study 39

* Not-acceptable studies were not included.
[2] These studies are considered supplementary.
[3] These studies were not submitted by the applicant (but available to the RMS e.g. from other procedures).

4.2.1.4 Acute toxicity: other routes

No other routes were tested.

4.2.2 Human information

Oral:

Cases of beta-cyfluthrin or cyfluthrin intoxication and signs of poisoning after oral ingestion are not known. Beta-cyfluthrin and cyfluthrin belong to the class of type II pyrethroid insecticides that are widely used, but there have been relatively few reports of systemic poisoning. These reports have, however, shown that pharmacotherapy is difficult and that the duration of poisoning can be unexpectedly long. Pyrethroids are ion channel toxins prolonging neuronal excitation, but are not directly cytotoxic. Two basic poisoning syndromes are seen. Type I pyrethroids produce reflex hyperexcitability and fine tremor. Type II pyrethroids produce salivation, hyperexcitability, choreoathetosis, and seizures. Both produce potent sympathetic activation. Systemic poisoning is difficult to control with anticonvulsants. Pentobarbitone, however, is surprisingly effective as therapy against systemic type II pyrethroid poisoning in rats, probably due to its dual action as a chloride channel agonist and a membrane stabilizer (study 43). Anyhow, it can be assumed that observations made after intoxication with other α-cyano-type II-pyrethroids are also applicable to beta-cyfluthrin. Patients with significant pyrethroid ingestion can present with severe symptoms and signs (Beasley and Wayne, National Poisons Centre, 2014; Table 24) which would constitute a medical emergency, and should be immediately referred to hospital for life support measures and ongoing monitoring. As

for other α-cyano-pyrethroids, there is no specific effective antidote. Seizures can be resistant to benzodiazepines and other pharmacotherapy; thiopental may be used in a hospital setting (Giampreti A, Lampati L, Chidini G, et al. Recurrent tonic-clonic seizures and coma due to ingestion of type I pyrethroids in a 19-month old patient. Clin Toxicol 2013; 51:497-500).

Table 24: Toxic effects of orally ingested pyrethroids

Mild pyrethroid toxicity	Moderate pyrethroid toxicity	Severe pyrethroid toxicity
Paresthaesia	CNS depression	Seizures
Nausea	Increased salivation	Coma
Headache	Fasciculations	Pulmonary oedema
Vomiting	Fever	Respiratory failure
Dizziness	Diaphoresis	
Fatigue	Blurred vision	
Anorexia		

Exposure via inhalation:

For determination of the tolerability following exposure by the inhaled and topical routes of an insecticide spray aerosol with cyfluthrin, a human volunteer study was designed (Ruddy et al, 1998; see also Chapter 4.2.2). Ten healthy male volunteers (2 exposure sessions of up to one hour (4 hours apart on the same day) with 5 subjects in each session) were exposed to two different concentrations of cyfluthrin dependent upon tolerability, , 4 hours apart on the same day. The administered concentrations were ≤ 0.1 mg cyfluthrin/m^3 air and 0.5-0.8 mg cyfluthrin/m^3 air.

The initial exposure concentration (≤ 0.1 mg cyfluthrin/m^3 air) was not tolerated and the higher exposure concentration was then cancelled. Laboratory analysis revealed that the initial actual concentration of the test substance had exceeded the defined concentration (ca. 0.2 mg cyfluthrin / m^3 air). The protocol was then amended to allow a further 5 subjects, at a later date, to be exposed to a lower concentration of 0.075 mg cyfluthrin/m^3 air for up to 1 h dependent upon tolerability. On this occasion, to alleviate anxiety, the subjects were exposed for 20 min to an atmosphere of placebo spray-can aerosol before exposure to the test substance (the group 1 volunteers (00I-005) completed the clinic phase of the study but were not exposed to the second exposure of the test substance. The group 2 volunteers (006-010) completed the study).

Only 2 of the 5 male volunteers in Group 1 tolerated the first exposure session for the defined period of 1 h. Adverse effects reported were: mild hyperaemia of the nasal mucosa (injection of blood vessels), moderate nasal irritation (running nose), mild irritation of the throat, coughing, sneezing, and watering eyes.

No clinically significant or drug related abnormalities in vital signs, EKGs or clinical laboratory tests were observed after 1-h exposure to airborne cyfluthrin concentrations of up to 0.2 mg/m^3. The observed events were all expected side effects of the test substance with reference to preclinical studies and observations in agrochemical workers and reflected irritation of the mucous membranes of the nose, throat, upper respiratory tract and eyes in order of frequency. The adverse effects were all self-limiting and resolved within minutes after cessation of exposure. It can be concluded that the initial concentration of ≤ 0.1 mg cyfluthrin/m^3 air appeared to be in the range of an irritant threshold concentration for humans (see Chapter 4.2.1 Proposal for classification with STOT SE 3).

Dermal:

Skin symptoms (paraesthesia) have been observed in people handling the active ingredient beta-cyfluthrin or cyfluthrin. Skin reactions such as pruritus, tautness and reddening of the facial skin, partial facial paraesthesia and signs of irritation in the oro-pharyngeal cavity or coughing, especially when concomitant with an elevated sensitivity, particularly to touch stimuli, may be signs of dermal

contact with exposure via inhalation to cyfluthrin. These symptoms may appear immediately or shortly after contact with the substance. They may last up to 24 (rarely to 48) hours and were often reported to be worsened by warmth (e.g. showering) (study 45).

The dermal sensations are direct and transitory effects on sensory nerve endings and not the result of a primary skin irritation. This conclusion is supported by the skin irritation study in rabbits with beta-cyfluthrin (study 42). There is no evidence for skin irritation as all mean scores for erythema, eschar formation as well as for oedema formation were 0. Therefore, and according to the "Guidance on the application of CLP criteria" (ECHA, 2012) no classification for skin irritation is needed.

Intravenous:

The American Journal of Emergency Medicine (Miller, 2014) reported that a 28-year-old man presented to the emergency department 20 minutes after injecting 20 mL of an insecticide containing 0.05% beta-cyfluthrin. The cause for the injection remained unknown. The man showed sinus tachycardia as the only symptom and was treated with an intravenous fluid bolus of 2000 mL (ingredients unknown). After 3 hours he fully recovered.

Dermal / Inhalation:

The Occupational Health Branch (OHB) of the California Department of Health Services (CDHS) conducts surveillance of work-related pesticide illness with support from the National Institute for Occupational Safety and Health (NIOSH) and the U.S. Environmental Protection Agency (EPA). On May 12, 2005, CDHS received a report from the California Department of Pesticide Regulation (CDPR) of a suspected pesticide incident in Kern County involving 27 farmworkers (age range: 21-61 years; median: 32.5 years) and six emergency responders (age range: 28-51 years; median: 33.5 years). CDHS investigated this incident by conducting a site visit, reviewing medical and meteorological records and interviewing affected workers, pesticide applicators, and the farmworker employer. Findings indicated that workers became ill from drift of a pyrethroid pesticide (cyfluthrin) that was being applied in a neighbouring field. Symptoms reported by the farmworkers were headache (96 %), nausea (89 %), eye irritation (70 %), muscle weakness (70 %), anxiety (67 %), and shortness of breath (64 %) (study 46).

4.2.3 Summary and discussion of acute toxicity

The lowest LD_{50} values determined in acceptable studies with beta-cyfluthrin were 77 mg/kg bw (acetone/peanut oil, study 22) in rats and 91 mg/kg bw (PEG 400) in mice (study 25). The lowest LD_{50} values determined in acceptable studies with cyfluthrin were 14.3 mg/kg bw (Cremophor/water) in rats (study 5) and 291 mg/kg bw (PEG 400) in mice (study 14). The proposal for classification for acute oral toxicity is based on cyfluthrin (solvent: Cremophor/water, study 5).

The LC_{50} values of beta-cyfluthrin and cyfluthrin were determined in rodents after exposure to dust. Based on the worst-case LC_{50} value determined in an acceptable inhalation study (study 36), the LC_{50} value in rats used for classification was 0.081 mg beta-cyfluthrin in ethanol/PEG 400/L air as mist (4h-exposure, head-nose only). The lowest rat LC_{50} value after dust exposure was 0.532 mg beta-cyfluthrin /L air (4h-exposure, head-nose only) (study 36).

The dermal toxicity of beta-cyfluthrin and cyfluthrin is very low. The lowest dermal LD_{50} value in rats determined in an acceptable study with beta-cyfluthrin was used for classification decision (>2000 mg/kg bw, solvent: PEG 400, study 42).

4.2.4 Comparison with criteria

The following table presents the relevant results used for acute toxicity classification and labelling and further lists the criteria given in the CLP regulation.

Table 25: Results of acute toxicity studies in comparison with CLP criteria

Toxicological result	CLP criteria
Oral ATE, rat: 14.3 mg cyfluthrin/kg bw (Vehicle: Cremophor (water)	Cat. 2 (H300): 5 < ATE ≤ 50 mg/kg (oral)
Dermal ATE, rat:>2000 mg beta-cyfluthrin/kg bw (Vehicle: PEG 400)	Cat. 4 (H312): 1000 < ATE ≤ 2000 mg/kg (dermal)
Inhalation ATE, rat: 0.081 mg beta-cyfluthrin/L air (highest attainable conc. 0.097 mg/L,Vehicle: ethanol/PEG 400; as misthead-nose only)	Cat. 2 (H330): 0.05 < ATE≤ 0.5 (dusts and mists)

4.2.5 Conclusions on classification and labelling

Based on the results listed above, the proposed classification and labelling for the rat oral ATE and inhalation ATE endpoint is

Acute Tox 2, H300 – Fatal if swallowed and

Acute Tox 2, H330 - Fatal if inhaled, respectively.

Beta-cyfluthrin does not meet the criteria for dermal toxicity classification.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute oral toxicity

The DS summarised data from six acute oral toxicity studies with beta-cyfluthrin and 20 with cyfluthrin. The lowest LD_{50} for beta-cyfluthrin of 77 mg/kg bw was found in a rat study using acetone/peanut oil as a vehicle (study 22). The DS noted that oral LD_{50} values of the two substances depend on the vehicle. Cremophor/water was found to be the vehicle leading to the lowest LD_{50} for cyfluthrin (out of the vehicles investigated), but the DS was not aware of any acute oral toxicity study with beta-cyfluthrin using Cremophor. The DS proposed Acute Tox. 2 with an ATE of 14.3 mg/kg bw based on a rat study with cyfluthrin using aqueous Cremophor as a vehicle (study 5).

Acute dermal toxicity

Three acceptable acute dermal toxicity studies are available for beta-cyfluthrin, reporting LD_{50} values of >2000 mg/kg bw or >5000 mg/kg bw. Acute dermal toxicity studies with cyfluthrin reported LD_{50} values of >5000 mg/kg bw, but were considered supplementary due to

insufficient reporting. The DS proposed no classification for acute dermal toxicity based on the studies with beta-cyfluthrin.

Acute inhalation toxicity

The DS summarised data from two acute inhalation toxicity studies with beta-cyfluthrin and eight studies with cyfluthrin. The DS proposed classification with Acute Tox. 2 based on an LC_{50} of 0.081 mg/L (mist) from a rat study with beta-cyfluthrin (study 36).

Comments received during public consultation

Comments on acute toxicity of beta-cyfluthrin were received from two MSCAs and one manufacturer.

While both MSCAs supported the DS's proposal, the manufacturer disagreed with the DS's assessment of acute oral toxicity, arguing that Cremophor is not a suitable vehicle in this case. The relevant OECD TGs (401, 420 and 423) indicate that the use of an aqueous solution/suspension/emulsion should be considered first, followed in order of preference by a solution/suspension/ emulsion in oil and then possibly solution in other vehicles. Cremophor is an emulsifier developed to enhance absorption of drugs and exaggerates the toxic potency of the test substance according to the manufacturer. Instead, they proposed to base the classification of both substances on the LD_{50} of 77 mg/kg bw observed in female rats administered beta-cyfluthrin in acetone/peanut oil (study 22). The DS replied that according to the Guidance on the application of the CLP criteria (ECHA, 2017) the lowest valid value should be the basis for classification, and retained their original position.

Additional key elements

In their comment during public consultation, the manufacturer provided results from a non-guideline acute oral toxicity study in Wistar rats with beta-cyfluthrin using Cremophor as a vehicle (Anonymous, 1986) yielding an LD_{50} of 11 mg/kg bw. The study report was included the plant protection product (PPP) dossier of beta-cyfluthrin. Details of the study are presented in the table below.

Acute oral toxicity study (Anonymous, 1986)	
Method	**Observations**
Substance: beta-cyfluthrin technical, batch 16001/85 Vehicle: Cremophor EL/distilled water Species and sex: rat, male Kind of application: oral, fasted Doses: 10.0, 11.2, 12.5, 16.0 mg/kg bw Application volume: 10 mL/kg bw No. of animals per dose group: 5 Post-treatment observation period: 14 days	Mortality: 10.0 mg/kg bw: 1 out of 5 11.2 mg/kg bw: 1 out of 5 12.5 mg/kg bw: 4 out of 5 16.0 mg/kg bw: 5 out of 5 All animals at all doses showed clinical signs of toxicity LD_{50}: 11 mg/kg bw (10.7–12.6)

Assessment and comparison with the classification criteria

Acute oral toxicity

Out of the vehicles tested, Cremophor yielded the lowest LD_{50} values for both beta-cyfluthrin and cyfluthrin. The rat LD_{50} for beta-cyfluthrin in Cremophor was 11 mg/kg bw compared to 77 mg/kg bw in acetone/peanut oil. Other vehicles (including PEG 400) led to higher values.

Beta-cyfluthrin is a strongly lipophilic substance (log K_{ow} ca. 6). RAC notes that according to the relevant OECD TGs, water and oil are generally preferred to other vehicles and that vegetable oils have been widely used for acute oral toxicity testing of pyrethroids. On the other hand, Cremophor is a surfactant and surfactants are found in PPPs containing pyrethroids. Thus, Cremophor cannot be dismissed as a vehicle for human hazard assessment. Therefore, RAC agrees to base the classification on studies where the substance was dissolved in aqueous Cremophor.

The lowest valid LD_{50} in a relevant species should generally be used as a basis for classification. **RAC proposes to classify beta-cyfluthrin for Acute Tox. 2; H300 with an ATE of 11 mg/kg bw** based on a rat acute toxicity study with beta-cyfluthrin using aqueous Cremophor as a vehicle (Anonymous, 1986).

Acute dermal toxicity

Three acute dermal toxicity studies, all OECD test guideline- and GLP-compliant, are available for beta-cyfluthrin (studies 40, 41, 42; rat, vehicle PEG 400 or xylene). They reported LD_{50} values of >2000 mg/kg bw or >5000 mg/kg bw.

As all available dermal LD_{50} values are above 2000 mg/kg bw, RAC agrees with the DS that **no classification is warranted for acute dermal toxicity**.

Acute inhalation toxicity

The lowest LC_{50} for beta-cyfluthrin was 0.081 mg/L (study 36; rat, head/nose only, vehicle ethanol/PEG 400; OECD TG 403, GLP). This LC_{50} value corresponds to Category 2 (0.05 < ATE ≤ 0.5 mg/L). Thus, **RAC agrees with the DS's proposal to classify beta-cyfluthrin for Acute Tox. 2; H330 with and ATE of 0.081 mg/L (dusts or mists).**

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

4.3.1 Non-human information

Teratogenicity studies with exposure via inhalation in rats (study 77 and 78) showed respiratory disturbances and bradypnoea due to irritative aerosol concentrations of cyfluthrin.

In an inhalation study for embryotoxic effects with cyfluthrin (study 78), a physiological maternal compensation mechanism (hypothermia with respiratory alkalosis) followed by reflex bradypnoea due to sensory irritation (see section reproductive toxicity/teratogenicity) was observed. At doses of 11.9 and 12.8 mg cyfluthrin plus oxygen/m^3 air clear signs of maternal toxicity occurred in the form of respiratory disturbances and hypoactivity in dams and a high-stepping gait and salivation at 11.9 mg/m^3 air only. No gross pathological findings were recorded at necropsy of any dose group (including the satellite groups).

The animals of the lower dose groups exhibited a concentration-dependent hypothermia and bradypnoea (hypoventilation) after the first exposure at levels of 0.46 mg/m³ air and above. .After the seventh exposure this hypothermia could still be determined in the high dose group only, being less severe in the group with oxygen substitution. In the 2.55 mg/m³ air dose group concentrations were tolerated without an effect on body weight gain. No signs of toxicologically significant neurological or sensorimotor changes (reflex tests) were seen. Comparing the findings from the groups with and without oxygen substitution permits the conclusion that the increase in the partial pressure of oxygen in the inhalation chamber produced an attenuation of the maternal toxic effects. There were no significant differences in the plasma cyfluthrin levels in the groups with and without oxygen substitution.

4.3.2 Human information

For determination of the tolerability following exposure by the inhaled and topical routes of an insecticide spray aerosol with cyfluthrin, a human volunteer study was designed (study 44). Ten healthy male volunteers (2 exposure sessions of up to one hour (4 hours apart on the same day) with 5 subjects in each session) were exposed to two different concentrations of cyfluthrin dependent upon tolerability. The administered concentrations were ≤ 0.1 mg cyfluthrin/m^3 air and 0.5-0.8 mg cyfluthrin/m^3 air.

The initial exposure concentration (≤ 0.1 mg cyfluthrin/m^3 air) was not tolerated and the higher exposure concentration was then cancelled. Laboratory analysis revealed that the initial actual concentration of the test substance had exceeded the defined concentration (ca. 0.2 mg cyfluthrin / m^3 air). The protocol was then amended to allow a further 5 subjects, at a later date, to be exposed to a lower concentration of 0.075 mg cyfluthrin/m³ air for up to 1 h dependent upon tolerability. On this occasion, to alleviate anxiety, the subjects were exposed for 20 min to an atmosphere of placebo spray-can aerosol before exposure to the test substance (the group 1 volunteers (00I-005) completed the clinic phase of the study but were not exposed to the second exposure of the test substance. The group 2 volunteers (006-010) completed the study).

Only 2 of the 5 male volunteers in group 1 tolerated the first exposure session for the defined period of 1 h. Adverse effects reported were: mild hyperaemia of the nasal mucosa (injection of blood vessels), moderate nasal irritation (running nose), mild irritation of the throat, coughing, sneezing, and watering eyes.

Table 26: Group 1 – Adverse Effects

Volunteer No.	Initial expose con. (mg cyfluthrin/m³ air)	Time of exposure	Adverse effect	Severity	Reversibility
1	0.2	60 min	Hyperaemia of nasal mucosa	Mild	yes
2	0.2	40 min	Hyperaemia of nasal mucosa; Nose running (clear mucous), Irritation of the throat	Mild/Moderate	yes
3	0.2	3 min	Coughing, Headache	Mild/Moderate	yes
4	0.2	60 min	Nose running, sneezing, eyes watering,	Mild	yes

Volunteer No.	Initial expose con. (mg cyfluthrin/m³ air)	Time of exposure	Adverse effect	Severity	Reversibility
			intermittent coughing		
5	0.09	25 min	Nose running, nasal muscosa injected	Mild	yes

All 5 volunteers in group 2 tolerated a 20 min exposure to placebo spray-can aerosol to alleviate anxiety before the second exposure session and no adverse events were reported. All 5 volunteers tolerated the second exposure session for 1 h and 5 adverse events that were considered to be 'definitely' related to the test substance were reported. A single volunteer had objective evidence of mild hyperaemia of the nasal mucosa.

Table 27: Group 2 – Adverse Effects

Volunteer No.	Initial expose con. (mg cyfluthrin/m³ air)	Time of exposure	Adverse effect	Severity	Reversibility
6	0.1	60 min	Nasal irritation	Mild	yes
7	0.1	60 min	Nasal irritation	Mild	yes
8	0.1	60 min	No adverse effects noted	-	-
9	0.1	60 min	Nose running, irritation at back of throat	Mild	yes
10	0.1	60 min	Irritation at back of throat	Mild	yes

No clinically significant or substance related abnormalities in vital signs, EKGs or clinical laboratory tests were observed after 1-h exposure to airborne cyfluthrin concentrations of up to 0.2 mg/m³. The observed effects were all expected side effects of the test substance with reference to preclinical studies and observations in agrochemical workers and reflected irritation of the mucous membranes of the nose, throat, upper respiratory tract and eyes in order of frequency. The adverse effects were all self-limiting and resolved within minutes after cessation of exposure. The initial concentration of 0.1 mg cyfluthrin/m^3 air appeared to be in the range of an irritant threshold concentration for humans.

Further impacts on people handling the active ingredient cyfluthrin (synthesis laboratory, manufacturing plant, formulation plant, toxicological laboratory) included signs of irritation in the oro-pharyngeal cavity, the eyes and skin effects (study 52-54).

Beside skin (paraesthesia) and eye symptoms, signs of irritation in the oro-pharyngeal cavity or coughing, were reported after inhalation/airborn exposure to cyfluthrin. These symptoms may appear immediately or shortly after contact with the substance, they may last up to 24 (rarely to 48) hours, and it was often reported to be worsened by warmth (e.g. showering). Likewise, symptoms reported from occupational airborne exposures were skin irritation and/or "Cold Burn", the paresthesias typical for skin contact to alpha-cyano pyrethroids, and airway irritation, in some cases provoking asthma-like reactions (no further details is reported) (study 45).

4.3.3 Summary and discussion of Specific target organ toxicity – single exposure

Medical data indicate the skin, eye, and the upper respiratory tract as main target organs towards

cyfluthrin. Symptoms like paresthesia of the skin, eye irritation, watering eyes, hyperaemia of the nasal mucosa, nasal irritation, mild irritation of the throat, coughing, sneezing and asthma-like reactions may occur after dermal/inhalation exposure of cyfluthrin. Animal data also showed respiratory disturbances and bradypnoea due to irritative aerosol concentrations of cyfluthrin.

The severity of the effects and the human health impact can indicate a borderline case for cyfluthrin classification criteria (e.g. even as STOT-SE, cat. 2). That is because the evidence in humans (asthma-like reactions, mild hyperaemia of the nasal mucosa, moderate nasal irritation, mild irritation of the throat, coughing, sneezing, and watering eyes) can also indicate a cytotoxic/inflammatory reaction.

It is also possible that these effects were related to the intrinsic sensory irritation of synthetic pyrethroids and would be out of the scope of STOT SE classification (Guidance on the Application of the CLP criteria, p. 434). However, there are no mechanistic and/or sufficient data details available to differentiate the local cytotoxic irritant from the sensory central reflex symptoms in the respiratory system (e.g. no appropriate histopathologic investigation of respiratory tract reported). Therefore, in order to make the user aware of the need for protection, the designation of Specific target organ toxicity-Single exposure, Cat. 3 May cause respiratory irritation (STOT SE; 3 H335) is proposed. Comparison with criteria:

Table 28: Categories for specific target organ toxicity-single exposure

Toxicological result	CLP criteria
Transient irritation of the mucous membranes (oro-pharyngeal cavity)	Transient target organ effects The category 3 only includes narcotic effects and respiratory tract irritation. These are target organ effects for which a substance does not meet the criteria to be classified in Categories 1 or 2. These are effects which adversely alter human function for a short duration after exposure and from which humans may recover in a reasonable period without leaving significant alteration of structure or function.

4.3.4 Conclusions on classification and labelling

Classification and labelling for respiratory irritation according to Regulation (EC) No 1272/2008 (GHS): STOT-SE 3, H335 (May cause respiratory irritation) based on data from cyfluthrin studies.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

The DS discussed respiratory disturbances in rat inhalation studies and human data on respiratory irritation. They proposed STOT SE 3; H335 mainly based on evidence of respiratory irritation in humans exposed to cyfluthrin or other pyrethroids (asthma-like reactions, mild hyperaemia of nasal mucosa, moderate nasal irritation, mild irritation of throat, coughing, sneezing, watering eyes; studies 44, 45, 52, 53, 54). The DS acknowledged the possibility that these symptoms may be related to sensory irritation and thus out of the scope of STOT SE classification. However, the available data were not considered sufficient to differentiate between cytotoxic or sensory irritation. Therefore, the

DS preferred to classify in order to make the user aware of the need for protection. The classification criteria for Categories 1 or 2 were not considered to be met since the symptoms were generally of short duration (lasting for up to 24 hours) and humans were assumed to be able to recover in a reasonable period of time without significant permanent alteration of structure or function.

Comments received during public consultation

Comments on the STOT SE classification of cyfluthrin and/or beta-cyfluthrin were received from four MSCAs and one manufacturer.

Three MSCAs supported the DS's proposal of STOT SE 3; H335. One of the MSCAs additionally proposed to consider classification for narcotic effects (STOT SE 3; H336) based on clinical signs such as tremors, ataxia and high-stepping gait in animal studies. The DS did not respond to this.

One MSCA proposed STOT SE 2 instead of STOT SE 3. In their opinion the symptoms observed in humans (asthma-like reactions, nasal irritation, irritation of the throat, coughing, sneezing, watering eyes) indicate cytotoxic reactions, the effects did not have a short duration after exposure and the symptoms could cause prolonged alteration.

The manufacturer presented a case against classification, arguing that there was no functional or histopathological evidence of cytotoxic irritation and/or inflammation in animal repeated exposure inhalation studies. The DS maintained that they did not find sufficient evidence to decide whether the symptoms observed in humans represented cytotoxic irritation or sensory irritation.

Assessment and comparison with the classification criteria

Respiratory tract irritation

Data on respiratory tract irritation are available from animal studies, a human volunteer study and occupationally exposed subjects.

Animal studies

Both acute and repeated exposure studies via inhalation in rats are available for cyfluthrin and beta-cyfluthrin.

The acute study 35 with beta-cyfluthrin reported hyperaemia of the visible nasal mucosa (as a clinical sign) from 11 mg/m^3 (LC_{50} ca. 90 mg/m^3; head/nose only, vehicle PEG/ethanol). However, no hyperaemia and no histopathological findings in the respiratory tract were observed at 24 mg/m^3 in a 4-w inhalation study with beta-cyfluthrin in the same strain (study 67; head/nose only, vehicle PEG/ethanol; the same author as of study 35). Decreased respiratory rate in study 67 was attributed to sensory irritation.

Human volunteer study (study 44)

Male volunteers were exposed to an insecticidal spray also containing cypermethrin (0.04%), piperonyl butoxide (0.22%), solvents (6.5%; acetone, kerosene), emulsifiers, fragrance, water and propellants. In the first experiment, only 2 out of 5 exposed subjects were able to tolerate exposure for 1 hour. Initial concentration of cyfluthrin in the first

experiment was ca. 0.2 mg/m^3. The findings included hyperaemia of nasal mucosa, running nose and coughing.

Human volunteer study, 1st experiment; initial concentration of cyfluthrin ca. 0.2 mg/m^3			
Subject no.	**Exposure duration (min)**	**Observations: subjective**	**Observations: objective**
1	60	No symptoms	Hyperaemia of nasal mucosa
2	40	Nasal irritation	Hyperaemia of nasal mucosa
		Nose running clear mucous	Nose running clear mucous
		Irritation of the throat	Normal
3	3	Coughing	Chest clear
4	60	Nose running, sneezing	Normal
		Eyes watering	Normal
		Coughing - intermittent	
5	25 (initial conc. 0.09 mg/m^3)	Nose streaming	Nasal mucosa more injected than previously

The experiment was then repeated with another group at an initial concentration of ca. 0.1 mg/m^3 of cyfluthrin. The subjects were pre-exposed to a placebo spray to alleviate anxiety. All five subjects tolerated the exposure for 1 hour as intended. A single volunteer had objective evidence of slight hyperaemia of the nasal mucosa.

Human volunteer study, 2nd experiment; initial concentration of cyfluthrin ca. 0.1 mg/m^3		
Subject no.	**Observations: subjective**	**Observations: objective**
6	Slight nasal irritation	Slight hyperaemia
7	Nasal irritation	Normal
8	No effects	
9	Irritation at back of throat	Normal
	Nose running	Normal
10	Slight irritation at back of throat	Normal

RAC notes that the study was not designed as a double-blind placebo control study. Further, it is not clear to which extent other ingredients of the mixture (e.g. piperonyl butoxide) contributed to the observed irritation. Given that (beta-)cyfluthrin causes strong sensory irritation in animals and paresthesia in humans, it is plausible that the respiratory irritation in study 44 was caused mainly by cyfluthrin.

Reports from occupationally exposed subjects

The DS informed, with reference to studies 52-54, that people handling cyfluthrin (synthesis laboratory, manufacturing plant, formulation plant, toxicological laboratory) reported signs of irritation in the oro-pharyngeal cavity and the eyes besides skin effects.

RAC, upon examination of these documents, found out that they report irritation of the eyes, skin, lips and genitals, but not of the respiratory tract.

Respiratory irritation from alpha-cyano pyrethroids can reportedly lead to asthma-like reactions (study 45). Unfortunately, no further details are available to RAC, which makes the information not possible to evaluate.

Additional information, not specifically on cyfluthrin but on pyrethrins and pyrethroids in general, can be found in the 'Agency for Toxic Substances and Disease Registry' (ATSDR) report (ATSDR, 2003). Some of the reported symptoms are indicative of irritation while severe asthmatic reactions from dermal and inhalation exposure to pyrethrins (*i.e.* constituents of natural pyrethrum extract) suggest a potential role of allergy.

According the CLP criteria, classification in Category 3 for respiratory tract irritation (CLP, Annex I, 3.8.2.2.1) is based primarily on symptoms of respiratory irritation in humans (e.g. redness, cough, pain, breathing difficulties). Subjective human observations could be supported by objective measurements (such as electrophysiological responses, biomarkers of inflammation). Ambiguous reports of simply 'irritation' shall be excluded as this term is commonly used to describe a wide range of sensations including smell, a tickling sensation or dryness, which are outside the scope of classification.

Animal data, such as relevant clinical signs of toxicity (e.g. dyspnea, rhinitis) and histopathological evidence of irritation (e.g. hyperaemia, oedema, minimal inflammation, thickened mucous layer), can be used as part of weight of evidence evaluation.

A STOT SE 3 classification for respiratory irritation can be applied only when more severe organ effects including in the respiratory system are not observed.

The Guidance on the application of the CLP criteria (CLP guidance, version 5.0, ECHA, 2017) further specifies that the generic term 'respiratory tract irritation' covers two different effects: 'sensory irritation' and 'local cytotoxic effects'. According to the CLP guidance, classification for STOT SE Category 3 for respiratory tract irritation is generally limited to local cytotoxic effects. In the plenary discussion, some RAC members expressed a view that the CLP guidance is unclear and contradictory in this regard, and questioned whether the CLP guidance should be followed in this case. Still, RAC agreed that the currently applicable CLP guidance should be followed, and that where it can be established that sensory irritation is the sole mode of action (MoA), the substance should not be classified.

For cyfluthrin, cough, hyperaemia of nasal mucosa (objective) and irritation of the nasal cavity and throat (subjective) were reported in humans after a single exposure (<1 hour) to concentrations of 0.1-0.2 mg/m^3 (study 44).

Clear evidence of respiratory tract irritation (bradypnoea) has been found in rat studies with (beta-)cyfluthrin at non-lethal concentrations. However, given the lack of histopathological findings in the respiratory tract up to 24 mg/m^3 (4-w study 67), these effects are considered to represent sensory, not cytotoxic irritation.

In summary, there is clear evidence of respiratory tract irritation from (beta-)cyfluthrin exposure in animals and some evidence of respiratory tract irritation in humans. As no histopathological changes in the respiratory tract were observed in a rat subacute study (study 67) up to high concentrations, (beta-)cyfluthrin-related respiratory tract irritation is considered to represent sensory, not cytotoxic irritation. Therefore, **classification for respiratory tract irritation is not warranted**.

Neurotoxicity

The available information indicates that the cause of deaths in acute toxicity studies with pyrethroids is neurotoxicity (ATSDR, 2003). The neurotoxic effects (e.g. abnormal gait, salivation) in repeat dose studies are considered to represent a series of acute intoxications. Clinical signs of neurotoxicity typically lasted for several hours after administration and resolved before the next dose (Anonymous, 1983; study 63).

The proposed acute oral toxicity classification (Acute Tox. 2; ATE = 11 mg/kg bw) is based on a rat gavage study using aqueous Cremophor as a vehicle. With Cremophor, clinical signs of neurotoxicity started close to doses associated with mortality (studies 61, 76; Anonymous, 1997a, 1999).

In rat gavage studies using PEG 400 clinical signs began from about 40 mg/kg bw/d (study 72; Anonymous, 1983) and mortality from 100 mg/kg bw (study 21).

In rat dietary studies clinical signs of neurotoxicity started from ca. 60 mg/kg bw/d (study 59 – symptoms already after the 1st dose; study 70). Dogs were more sensitive with effects present already around 10 mg/kg bw/d (studies 60 and 63). Lethal doses via dietary route are not known.

Acute dermal toxicity studies reported no mortality up to 2000 mg/kg bw, a single mortality in a single study was observed at 5000 mg/kg bw (study 40). Clinical signs indicative of neurotoxicity (e.g. splayed gait) were observed from 1000 mg/kg bw (studies 40 and 41).

The proposed classification for acute inhalation toxicity is Acute Tox. 2 (ATE = 0.081 mg/L; vehicle ethanol/PEG 400). The information on the threshold for neurotoxicity in the acute studies available to RAC is limited. Increased activity after exposure was reported in subacute studies at 0.024 and 0.047 mg/L (study 67; Anonymous, 1989), which is relatively close to the ATE.

As to human data, signs of mild acute pyrethroid poisoning include dizziness, headache, and nausea, in addition to paresthesia. Higher levels of exposure to pyrethroids result in additional clinical signs such as lethargy, muscle twitches, and mild disturbance of consciousness. Even higher exposure levels may result in convulsive attacks and coma, and these severe effects may last for several weeks (ATSDR, 2003, p. 69).

Paresthesia observed in humans exposed to cyfluthrin (studies 52, 53, 54) and other pyrethroids (ATSDR, 2003), although not a severe effect by itself, is also a manifestation of neurotoxicity and may be viewed as additional support for a STOT SE classification.

Based on the available animal data on (beta-)cyfluthrin and human data on pyrethroids, RAC concludes that the interval between the threshold for neurotoxicity and lethal doses is sufficiently large at least for some routes of exposure to justify classification with STOT SE. In addition, no acute toxicity classification is proposed for the dermal route while neurotoxicity after dermal exposure was observed in rats.

As the clinical signs in animals occurred at or below 300 and 1000 mg/kg bw after oral and dermal exposure respectively, **classification in Category 1 is considered appropriate**. Classification in Category 1 is further supported by human data on pyrethroids.

RAC concludes that classification for STOT SE 1; H370 (nervous system) is justified based on clinical signs of neurotoxicity occurring in some cases significantly below lethal doses.

4.4 Irritation

4.4.1 Skin irritation

4.4.1.1 Non-human information

Beta-cyfluthrin is not irritating to the skin. This result is supported by skin irritation studies with cyfluthrin.

Table 29: Summary table of relevant skin irritation studies with beta-cyfluthrin*

Parameter	Species	Vehicle	Result	Reference
Skin irritation (GLP: yes, OECD 404)	Rabbit (3 female albino Esd:NZW rabbits)	Water beta-cyfluthrin (batch no.: FFEBCTQ043, purity: 99.2 %)	Non-irritant	study 49 †

* Not-acceptable studies were not included.
†Key study

Table 30: Summary table of relevant skin irritation studies with cyfluthrin*

Parameter	Species	Vehicle	Result	Reference
Skin irritation (GLP: no, unpublished)	Rabbit (Albino Japanese) 6 females	Undiluted (cyfluthrin lot no. Eg 3/81, purity: 95%)	Non-irritant[2,3]	study 47
Skin irritation (GLP: no, unpublished)	Rabbit (White New Zealand) 6 males	Unclear (cyfluthrin batch no. 16001/79, purity: 83.6%)	Non-irritant[2,3]	study 48

* Not-acceptable studies were not included.
[2] The study is considered supplementary.
[3] These studies were not submitted by the applicant (but available to RMS e.g. from other procedures)

4.4.1.2 Human information

Skin symptoms (paraesthesia) have been observed in people handling the active ingredient cyfluthrin. Skin reactions such as pruritus, tautness and reddening of the facial skin, partial facial paraesthesia and signs of irritation in the oro-pharyngeal cavity or coughing, especially when concomitant with an elevated sensitivity, particularly to touch stimuli, may be signs of dermal contact with or inhalation exposure to cyfluthrin. These symptoms may appear immediately or shortly after contact with the substance, they may last up to 24 (rarely to 48) hours, and it was often reported to be worsened by warmth (e.g. showering). Likewise, symptoms reported from airborne exposures were skin irritation and/or "Cold Burn", the paresthesias typical for skin contact to alpha-cyno pyrethroids, and airway irritation, in some cases provoking asthma-like reactions. These too, are well known for pyrethroids (study 45).
In order to make the user aware of the need for protection, the designation of STOT-SE 3 H335 'May cause respiratory irritation' according to Regulation (EC) No 1272/2008 is proposed (see Chapter 4.2.1).

4.4.1.3 Summary and discussion of skin irritation

Beta-cyfluthrin is not irritating to the skin.

The dermal sensations are direct and transitory effects on sensory nerve endings and not the result of a primary skin irritation. This conclusion is supported by the skin irritation study in rabbits (study 49). There is no evidence for skin irritation as all mean scores for erythema, eschar formation as well as for oedema formation were 0. Therefore, and according to the "Guidance on the Application of CLP criteria" (ECHA, 2012) no classification for skin irritation is needed.

4.4.1.4 Comparison with criteria

The following table presents the critical results for skin irritation used for classification and labelling and further list the criteria required from CLP regulation.

Table 31: Results of skin irritation tests in comparison with CLP criteria*

Toxicological result	CLP criteria
Mean erythema and oedema scores (24-72 h): 0.0 and 0.0, respectively (no animal ≥2.3). (study 49)	Irritating to skin (Category 2, H315): at least in 2/3 tested animal a positive response of: Mean value of ≥2.3-≤4.0 for erythema/eschar or for oedema

* Only acceptable studies were used for classification.

4.4.1.5 Conclusions on classification and labelling

Based on the results above, no classification regarding skin irritation/corrosion is triggered.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification based on a negative skin irritation study in rabbits with beta-cyfluthrin where no evidence of skin irritation was observed (study 49). Human reports of paresthesia, typical for alpha-cyano pyrethroids, were considered to represent a direct effect on sensory nerve endings rather than a primary skin irritation.

Comments received during public consultation

Comments on the skin irritation classification of cyfluthrin and/or beta-cyfluthrin were received from three MSCAs, all in support of the DS's proposal.

Assessment and comparison with the classification criteria

One OECD test guideline- and GLP-compliant *in vivo* study is available for beta-cyfluthrin (study 49). The substance was applied as a powder moistened with water. All mean scores for erythema/eschar and oedema were 0.

Two *in vivo* studies are available for cyfluthrin. In study 47 the substance was applied undiluted as a viscous liquid for 24 h (OECD TG 404: 4 h), the applied amount was 0.1 mL (OECD TG 404: 0.5 mL). Slight erythema was noted in 1 out of 4 animals 24 h after patch removal and disappeared by the 72 h time point. The study is considered negative. The available information on study 48 is rather limited; the study was negative according to the CLH report.

RAC agrees with the DS that no classification for skin corrosion/irritation is warranted.

4.4.2 Eye irritation

4.4.2.1 Non-human information

Table 32: Summary table of relevant eye irritation studies with beta-cyfluthrin*

Parameter	Species	Vehicle	Result	Reference
eye irritation (GLP: no, OECD 405)	Rabbit (3 male albino HC:NZW rabbits)	Unclear (beta-cyfluthrin batch no.: 16002/84, purity: 98.5 %)	non-irritant[1]	Study 50 †
eye irritation (GLP: yes, OECD 405)	Rabbit (3 female albino HsdIf:NZW rabbits)	Undiluted (beta-cyfluthrin batch no.: FFEBCTQ043, purity: 99.2 %)	non-irritant[1]	Study 51 †

* Not-acceptable studies were not included.
[1] Slight effect, does not fulfil the criteria for classification.
†Key study

Table 33: Summary table of relevant eye irritation studies with cyfluthrin*

Parameter	Species	Vehicle	Result	Comment	Reference
eye irritation (GLP: no, unpublished)	Rabbit (Albino Japanese) 12 female	Undiluted (cyfluthrin lot no. Eg 3/81, purity 95%)	irritant[1,2,3]	-cyfluthrin used in melted state -observation period only 3 days (TG 404, 1981) -only 100 µl instead of 500 µl tested (TG 404, 1981) -24 h instead of 4 h exposure (TG 404, 1981) -skin observed after 24 h and 72 h (not 48 h) (TG 404, 1981)	Study 47
eye irritation (GLP: no, unpublished)	Rabbit (White New Zealand) 3-5 males	Unclear (cyfluthrin batch no. 16001/79, purity: 83.6%)	irritant[2,3,4]	-24 h instead of 4 h exposure (TG 404, 1981) -material section refers to document which is not available -some details remain unclear (e.g. whether substance is moistened)	Study 48

* Not-acceptable studies were not included.
[1] From the data given it remains unclear whether from today's perspective the outcome would be positive, too.
[2] The study is considered supplementary.
[3] These studies were not submitted by the applicant (but available to RMS e.g. from other procedures).
[4] If gradings are comparable with today, the substance would be considered as not irritating to eyes (based on mean scores after 24, 48, 72 h).

The following results were obtained with beta-cyfluthrin (study 50): Slightly irritating effects were noted after 1 h and 24 h to the conjunctivae (redness, swelling, tear flow). Considering the time points 24, 48 and 72 h, the mean values for corneal opacity and iritis were 0 and for all conjunctival parameters not above 1.3. All effects observed were reversible. Beta-cyfluthrin showed a slightly irritating effect on the eye. According to the CLP criteria, beta-cyfluthrin is not to be classified as irritating to eyes.

Table 34: Test for irritant/corrosive impact of the test compound beta-cyfluthrin on the rabbit's eye (study 50)

Animal no.	Grade after#																Mean value after			
	24h				48h				72h				7d				24h, 48h, 72h			
	CO	IR	CR	COE	CO	IR	CR	COE	CO	IR	CR	COE	CO	IR	CR	COE	CO	IR	CR	COE
J1	0	0	2	2	0	0	1	1	0	0	1	0	0	0	0	0	0	0	1.3	1
M27	0	0	2	1	0	0	1	1	0	0	1	0	0	0	0	0	0	0	1.3	0.7
M24	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0.7	0.3

CO = corneal opacity, IR = iritis, CR = conjunctival redness, COE = conjunctival oedema.

In addition, in the 2nd eye irritation study with beta-cyfluthrin (study 51) similar findings were noted: Three rabbits showed conjunctival redness (Grade 1-2) and 2/3 animals chemosis (Grade 1) after 24 h post application. In one animal grade-1 conjunctival erythema persisted until 48 h post application. None of the animals showed signs of eye irritation at 72 h post application. Iris and cornea were not affected by treatment at any time point. Thus, beta-cyfluthrin is not irritating to eyes.

Table 35: Test for irritant/corrosive impact of the test compound beta-cyfluthrin on the rabbit's eye (study 51)

Animal no.	Grade after#												Reversible after				Mean value after			
	24h				48h				72h				x days				24h, 48h, 72h			
	CO	IR	CR	CC	CO	IR	CR	CC	CO	IR	CR	CC	CO	IR	CR	CC	CO	IR	CR	CC
1	0	0	2	1	0	0	1	0	0	0	0	0	n.a.	n.a	3	2	0	0	1	0.3
2	0	0	1	0	0	0	0	0	0	0	0	0	n.a	n.a	2	1*	0	0	0.3	0
3	0	0	2	1	0	0	0	0	0	0	0	0	n.a	n.a	2	2	0	0	0.7	0.3

CO = corneal opacity, IR = iritis, CR = conjunctival redness, CC = chemosis conjunctivae, na: not applicable
* = in respect of the result 1 h post application.

Eye irritation studies with cyfluthrin showed a minimally irritating effect in Japanese rabbits (study 47, see Table 33). It was assumed that the substance has some sensory irritant effect, because after treatment animals rubbed both eyes with both paws. Also technicians felt a sense of irritation after handling of the test substance. Observation and scoring for cornea, iris and conjunctivae were examined at 1, 3, 6, 24 hours and 2, 3, and 7 days after treatment. The treatment had no effect on the cornea. In the non-irrigation group, hyperemia of the iris was seen in two animals at 1 hour after the application. The effect disappeared after 6 hours post application. Redness, chemosis and secretion of the conjunctiva were seen regardless of non-irrigation or irrigation. Cyfluthrin was considered to be mildly irritating to the eye. Anyhow, a conclusion for classification cannot be drawn from this study as it remains unclear whether from today's perspective the outcome would be evaluated as positive (e.g. scoring not consistent with OECD TG and observation time < 21 days).

Likewise, in study 48, redness of the conjunctivae was noted up to 72 hours post application, slight chemosis up to 24 hrs after application. The findings were all reversible. If gradings are comparable

with today, the substance would be considered as not irritating to eyes (based on mean scores after 24, 48, and 72 h). Therefore, based on the severity and the reversibility of these findings classification is not warranted.

4.4.2.2 Human information

During the production period since 2005 two accidents with beta-cyfluthrin occurred in workers, resulting in facial and eye irritation. Symptoms resolved very quickly. No further consultations of the Medical Department due to handling or contact with beta-cyfluthrin were required (study 45).

Skin and eye symptoms have been observed in workers in connection with the handling of cyfluthrin (Study 52, 53, 55, 56). The observations relate to people who have handled the active substance (synthesis laboratory, manufacturing plant, formulation plant, toxicological laboratory). Symptoms included skin reactions such as pruritus, tautness and reddening of the facial skin, partial facial paraesthesia, signs of irritation in the oro-pharyngeal cavity and the eyes. After onset of the irritation signs, an elevated sensitivity, particularly to touch stimuli, was observed. The effects were reversible within a few hours.

No health problems or changes in well-being were mentioned in connection with handling of cyfluthrin when the work rules were observed. Conclusions were drawn that by precautionary measures such as the wearing of protective clothing and avoidance of direct and indirect contamination of the relevant skin areas and the eyes, effects of cyfluthrin can be prevented.

Extensive training, more sophisticated plant technology and stricter protective measures are needed when handling the active ingredient cyfluthrin as a dust formulation. Even slight contact of dust with the skin or mucosa of the eye, initially unnoticed, results in an unpleasant irritation and burning sensation at the site of contact within a few hours (first signs generally occur after showering) (study 54).

In a human volunteer study, inhalation exposure to different concentrations of cyfluthrin resulted in irritation of the eyes and other adverse effects (irritation of the mucous membranes of the nose, upper respiratory tract and throat) (study 44) (see also Chapter 4.3.1 Proposal for classification with STOT SE 3).

The Occupational Health Branch (OHB) of the California Department of Health Services (CDHS) conducts surveillance of work-related pesticide illness with support from the National Institute for Occupational Safety and Health (NIOSH) and the U.S. Environmental Protection Agency (EPA). In 2005, CDHS received a report from the California Department of Pesticide Regulation (CDPR) of a suspected pesticide incident in Kern County involving 27 farmworkers (age range: 21-61 years; median: 32.5 years) and six emergency responders (age range: 28-51 years; median: 33.5 years). After spraying of a cyfluthrin containing pesticide the following symptoms were reported by the exposed farmworkers: headache (96 %), nausea (89 %), eye irritation (70 %), muscle weakness (70 %), anxiety (67 %), and shortness of breath (64 %). Illness symptoms were not reported by the applicators, who were wearing appropriate protective equipment (study 46) (see also chapter 4.1.2 Human information – Dermal / Inhalation).

4.4.2.3 Summary and discussion of eye irritation

Eye irritation studies in rabbits revealed slight or no eye irritating effects and do not trigger a proposal for classification. Human data showed some slight, reversible eye symptoms on different occasions, mainly in connection with the handling of beta-cyfluthrin and cyfluthrin. No former proposal on classification for eye irritation was made.

4.4.2.4 Comparison with criteria

The following table compares the critical results for eye irritation used for classification and labelling and the criteria given in the CLP regulation.

Table 36: Results of eye irritation studies in comparison with CLP criteria*

Toxicological result	CLP criteria
Mean score (24-72 h): Corneal opacity: 0.0 (no animal ≥1) Iris leson: 0.0 (no animal ≥1) Conjuntival redness: not above 1.3 (no animal ≥2) Oedema of the conjunctivae (chemosis): not above 1 (no animal ≥2) (study 50) Mean score (24-72 h): Corneal opacity: 0.0 (no animal ≥1) Iris leson: 0.0 (no animal ≥1) Conjuntival redness: not above 1.0 (no animal ≥2) Oedema of the conjunctivae (chemosis): not above 0.3 (no animal ≥2) (study 51)	Irritating to eyes (Category 2, H319): at least in 2/3 tested animal a positive response of: corneal opacity: ≥1 and/or iritis: ≥1 and/or conjunctival redness: ≥2 and/or conjunctival oedema (chemosis): ≥2 Calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material, and which fully reverses within an observation period of 21 days.

* Only acceptable studies were used for classification.

4.4.2.5 Conclusions on classification and labelling

Based on the results above, no classification regarding eye irritation/corrosion is triggered.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification for serious eye damage/irritation based on two *in vivo* studies with beta-cyfluthrin (study 50 and 51) reporting mild eye irritation not meeting the classification criteria.

Comments received during public consultation

One MSCA supported the DS's proposal.

Assessment and comparison with the classification criteria

Two OECD test guideline- and GLP-compliant *in vivo* studies are available for beta-cyfluthrin (studies 50 and 51). The maximum mean scores for conjunctival redness or oedema were 1.3 and 1 in study 50 and 51 respectively (a mean score of ≥ 2 in 2 out of 3 animals triggers classification); the effects were reversible. No corneal opacity or iritis was present. The studies are considered negative.

Two pre-/non-guideline *in vivo* eye irritation studies are available for cyfluthrin (study 47 and 48), both reporting mild eye irritation. Study 47 is not suitable for classification purposes as it employed a different grading system from that recommended in the OECD TG 405. The pre-guideline study 48 can be considered negative provided the grading system was comparable to that used under CLP.

RAC agrees with the DS that no classification for eye damage/irritation is warranted.

4.4.3 Respiratory tract irritation

On the basis of the findings mentioned above, it is proposed to also classify beta-cyfluthrin for respiratory irritating properties (see Chapter 4.3: Specific target organ toxicity – single exposure (STOT-SE)).

4.5 Corrosivity

Beta-cyfluthrin does not meet the criteria for skin/eye irritation/corrosion. Thus, no classification is triggered.

4.5.1 Conclusions on classification and labelling

Based on the results of studies on acute dermal toxicity, skin and eye irritation, no classification regarding skin/eye corrosion is triggered.

4.6 Sensitisation

4.6.1 Skin sensitisation

4.6.1.1 Non-human information

Table 37: Summary table of relevant skin sensitisation studies with beta-cyfluthrin*

Parameter	Species	Vehicle	Result	Reference
Skin sensitization (Buehler Patch Test) (GLP: yes, OECD 406)	Guinea pig (Crl:HA) (10 females)	cremophor/saline beta-cyfluthrin (batch no.: FFEBCTQ043, purity: 99.2)	no sensitizer[1]	study 58

* Not-acceptable studies were not included.
[1] The study is considered supplementary.

Table 38: Summary table of relevant skin sensitisation studies with cyfluthrin*

Parameter	Species	Vehicle	Result	Comment	Reference
Skin sensitization (GLP: yes, Magnussen Kligman Test)	Guinea pig (Hsd/Win:DH) 50 male	PEG 400 (cyfluthrin batch no. 380368010, purity 96.2%) -Intraderm. ind.: 5 %	no sensitizer[1]	-unclear why dose-range-finding study was not extended to higher concentrations	study 57 †

		-Topical Ind.: 50 % -Challenge: 50 % and 25 %		(TG 406 1992)	

* Not-acceptable studies were not included.
†Key study

No evidence of a skin-sensitizing potential was found in a Buehler Patch Test with beta-cyfluthrin (study 58) and a Magnussen Kligman Test in guinea pigs with cyfluthrin (study 57). The Buehler Patch Test with beta-cyfluthrin was considered supplemental based on the following deviations of OECD-Guideline no. 406 (adopted in July 17, 1992):

1. Dose-range-finding studies were performed in order to find the dose for sensitization induction and challenge. OECD-Guideline no. 406 requires the highest dose to cause mild irritation for the induction exposure. For challenge exposure the highest non-irritating dose should be applied. A test item concentration of 66.6 % was chosen for the induction and challenge procedure even though no skin reaction was observed in the whole pilot study. This concentration did not show a mild irritation (for induction) and it is unclear whether this concentration matches the highest non-irritating dose (for challenge).Therefore, it remains questionable why the dose-range-finding study was not extended to higher concentrations above 66.6 % to investigate possible skin irritating effects at higher concentrations.
2. Although the test for skin sensitisation was conducted with a concentration of 66.6 %, both analyses for stability and homogeneity were performed with 0, 1 and 40 % but not with 66.6 % of the test item. Neither a rationale for this study deviation nor the method of these analyses was given.
3. Occlusive conditions were neither claimed nor documented for the main study.
4. This Buehler Patch Test was conducted with three applications only. Nine applications are considered valid for the evaluation of skin sensitization (EFSA Handbook for the experts' meetings, Section 2: Mammalian toxicology, 2010).

4.6.1.2 Human information

No information on skin sensitisation in humans is available.

4.6.1.3 Summary and discussion of skin sensitisation

Results of the GPMT (Vohr, 1994) (5% of animals with erythema at >1% intradermal induction dose) and the absence of skin effects in the Buehler test (study 58) do not show evidence of a skin-sensitizing potential.

4.6.1.4 Comparison with criteria

Table 39: Results of skin sensitisation tests in comparison with CLP criteria

Toxicological result	CLP criteria
Intradermal induction 5% Cyfluthrin (in PEG 400) Topical induction: 50% Cyfluthrin (in PEG 400) Challenge: 25% and 50% Cyfluthrin (in PEG 400) No skin reaction at 48 h after challenge; 1/20 animals showed skin reddening at 72 h after challenge (study 57)	Category 1B (H317): ≥30% to <60% responding at >0.1% to ≤1% intradermal induction dose or ≥30% responding at >1% intradermal induction dose
There were no skin effects in the animal of the test item	

Toxicological result	CLP criteria
group and the control group during the three induction treatments. The challenge with the 66.6% test item paste did not lead to skin effects in the animals of the test item group and in the control group. The study was considered supplemental (study 58).	Category 1B (H317): ≥15% to <60% responding at >0.2% to ≤20% topical induction dose or ≥15% responding at >20% topical induction dose

4.6.1.5 Conclusions on classification and labelling

Beta-cyfluthrin does not meet the criteria for skin sensitization. Thus, no classification is triggered.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS proposed no classification based on a negative Guinea Pig Maximisation Test (GPMT) with cyfluthrin (study 57) and a negative Buehler test with beta-cyfluthrin (study 58). However, they pointed out several supposed deficiencies:

- Lack of justification why higher concentrations (than 50% in the GPMT and 66% in the Buehler assay) were not tested;
- The Buehler test was conducted with three applications instead of nine;
- Occlusive conditions were not claimed nor documented for the main experiment in the Buehler test;
- Stability and homogeneity was documented for 40% but not 66% test item in the Buehler test, analytical method was not described.

Comments received during public consultation

One MSCA commented on beta-cyfluthrin and considered both studies (57 and 58) inadequate due to the deficiencies mentioned by the DS. No comments were received on cyfluthrin.

Assessment and comparison with the classification criteria

GPMT with cyfluthrin (study 57)

The study was conducted under GLP and according to OECD TG 406. The test substance group comprised 20 males. Two negative control groups consisted of 10 males each. There was no concurrent positive control group; reliability was periodically checked using 2-mercaptobenzothiazole.

The test substance, described as thick brown oil, was dissolved immediately prior to treatment in PEG 400 at 70°C to yield a solution. Stability was analytically verified. A concentration of 5% was used for intradermal induction, 50% for topical induction (1 week later) and 50% and 25% for challenge (21 days after the first induction). No pre-test on

irritant effects was performed. One day before topical induction animals were treated with 10% sodium lauryl sulphate in vaseline.

No skin reaction was seen in any animal in the control group. No skin reaction was seen in any animal of the test group at 48 h. At 72 h, one animal (out of 20) showed slight skin reddening at the challenge concentration of 50%.

RAC notes that the robust study summary (from the biocidal dossier) does not provide any explanation as to why higher concentrations were not tested. As the substance was a liquid, it could have been tested neat. On the other hand, the high viscosity and high lipophilicity (log K_{ow} ca. 6) of cyfluthrin are likely to hinder dermal uptake. Thus, solubilisation in an agent such as PEG 400 can be seen as a step increasing dermal uptake and thereby sensitivity of the method, rather than a deficiency.

Buehler test with beta-cyfluthrin (study 58)

The study was conducted under GLP and according to the OECD TG 406. The test substance group consisted of 20 animals, and 10 animals were used as negative controls. Reliability of the method was confirmed with alpha hexyl cinnamic aldehyde (25% and 45% of the animals exhibited dermal reactions after the first and second challenge, respectively).

The test substance, being a solid, was applied as a paste in Cremophor EL/saline (500 mg of test item mixed with 0.25 mL of the vehicle, *i.e.* ca. 66%). Three inductions took place at approximately weekly intervals. The challenge was performed 13 days after the last induction.

The substance did not induce any skin effect upon challenge in the test item group or in the control group (all scores 0).

RAC has not identified any critical deficiencies compromising validity of the study. It is considered plausible that a test concentration of 66% is near the highest attainable concentration for a solid in a paste. Three is the number of inductions required by OECD TG 406. RAC does not suspect the substance to be unstable at 66% when it was found to be stable at 40%. Occlusive conditions are mentioned for the pilot tests in the study report, so they are likely to have been applied also in the main test. Consequently, RAC considers the study adequate.

RAC concludes that no classification for skin sensitisation is warranted.

4.6.2 Respiratory sensitisation

No data available.

4.7 Repeated dose toxicity

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Table 40: Summary table of relevant repeated dose oral toxicity studies with beta-cyfluthrin

Study	Dose levels	Test substance	NO(A)EL [mg/kg bw/d]	Targets / Main effects	Reference
28-day, gavage; Wistar rat (4-week recovery) (GLP: yes, OECD 407)	0-0.25-1-4-16 mg/kg bw/d (5 males and 5 females/group)	Beta-cyfluthrin batch no.: 16002/84, purity: 98.5% Vehicle: Cremophor/water	1	≥4 mg/kg bw/d: Mortality, clinical signs, reduced bw development, increased liver weight	Study 61 †
90-day, feeding; Wistar rat (GLP: yes, OECD 408)	Males: 2.3, 9.5, 38.9/37* mg/kg bw/d Females: 2.5, 10.9, 42.4/43* mg/kg bw/d * Recovery (correspond to: 0, 30, 125 and 500 ppm) (15 males and 15 females/group)	Beta-cyfluthrin batch no.: 16001/85, purity: 99.7% no vehicle (covered in basal diet)	9.5/10.9 (125 ppm)	≥500ppm: Mortality, clinical signs, reduced bw and water intake, skin lesions, reduced red blood cell parameters, increased calcium levels in urine	Study 62 †
90-day, feeding; beagle dog (GLP: yes, OECD 409)	0-0.4-2.4-14 mg/kg bw/d (correspond to 0-10-60-360 ppm) Beagle dogs (4 males and 4 females/group)	Beta-cyfluthrin batch no.: 16001/85, purity: 99.7% no vehicle (covered in basal diet)	2.4 22.1 mg/animal/d (60 ppm)	14 mg/kg bw/d: Motor disturbances (hind limb), vomiting, diarrhea, reduced bw	Study 63 †

†Key study

Table 41: Summary table of relevant repeated dose oral toxicity studies with cyfluthrin*

Study	Dose levels	Test substance	NO(A)EL [mg/kg bw/d]	Targets / Main effects	Reference
90-day oral (feeding) toxicity in rats (GLP: no, Partly OECD 408)	0-100-300-1000 ppm (corr. to 6.21-18.98-60.90 mg/kg bw/d males, 7.29-21.22-68.47 mg/kg bw/d females) Sprague-Dawley rats (28 males and 28 females/group)	Cyfluthrin, batch no.: 816170019, purity: 95% no vehicle (covered in basal diet)	100 ppm (6.21 mg/kg bw/d)	≥300ppm: Gait abnormalities, salivation, slight axonal degeneration of sciatic nerve (reversible)	Study 59 †
12-month, feeding, Beagle dog (GLP: yes, OECD 452)	0-1.36-2.43-10.64-15.47 mg/kg bw/d in males 0-1.46-3.61-10.74-17.99 mg/kg bw/d in females (corr. to 0-50-100-360-640/500 ppm) Beagle dogs (4 males and 4 females/group)	Cyfluthrin batch no.: 4030059/BF9340-71, purity: 94.8-95.1% no vehicle (covered in basal diet)	2.43 / 3.61 mg/kg bw/d (100 ppm)	640/500 ppm: Premature sacrifice for welfare reasons ≥360 ppm: reduced bw, neurological disorders (gait abnormalities)	Study 60 †

* Not-acceptable studies were not included.
†Key study

In short-term toxicity experiments in rats and dogs oral administration of beta-cyfluthrin or cyfluthrin led to similar adverse effects: increased mortality, general behavioural disturbances, motor disturbances, lower body weight development, choreoathetotic signs, vomiting and diarrhea. No relevant effects on haematological, clinico-chemical and urine analytical parameters were detected. With the exception of a 3-month oral study in rats in which a reversible slight axonal degeneration was reported in some rats dosed with 1000 ppm (60.9 mg/kg bw/d) (study 59, see Table 44), gross or histopathological investigations did not afford any evidence of specific organ or tissue damage. This concerned also the tissues (nerve, muscle, eye) which were investigated in detail in a 28-day study on rats with beta-cyfluthrin (study 61).

A slight increase in liver weight, noticed in the 4-week rat study with beta-cyfluthrin (study 61) was not observed in a 13-week rat study at a higher dose of beta-cyfluthrin (study 62, see

Table 43). Alterations (clinical signs, reduced body weight development, increased liver weight) during the course of 4-week test substance exposure were reversible in a recovery period without test substance intake.

The resulting NOAEL of 125 ppm in the 13-week study on rats with beta-cyfluthrin, corresponding to 9.5 mg/kg bw/d in male and 10.9 mg/kg bw/d in female rats was based on mortalities, clinical signs and a reduction of body weight gain at the next higher dose (500 ppm).

In a 90-day study on dogs with beta-cyfluthrin (study 63, see Table 45) the NOAEL of 60 ppm, equal to 2.4 mg/kg bw/d, was based on motor disturbances, vomiting, diarrhea in males and females and a reduced body weight gain in females at the next higher dose of 360 ppm.

A 12-month feeding study in dogs with cyfluthrin (study 60, see table below) revealed slight to severe motor disturbances, vomiting, diarrhea and a reduction in body weight gain at ≥360 ppm (10.6-18 mg/kg bw/d). The study revealed a NOAEL of 100 ppm (2.4/3.6 mg/kg bw/d). This study supersedes the 12-month feeding study in dogs (study 64, see Table 46) which was considered not acceptable.

Table 42 Detailed findings in study 60

Detailed study findings							
Endpoint	**Sex**	**Concentration (ppm)**					**Comment**
		0	**50**	**100**	**360**	**500/640**	
No. animals/ group	Male/Female	4/4	4/4	4/4	4/4	4/4	
Mortalities	Male/Female	1/1	0/0	0/0	0/0	0/1	Control animals died from asymptomatic idiopathic epilepsy. High dose animal was sacrificed due to a compound-related neurologic condition.
Clinical signs							
Neurotoxicity signs (%)	Combined sexes	0	0	0	7 (87)	8 (100)	
Neuromuscular condition (%)	Combined sexes	1 (12)	0	0	0	2 (25)	
Body weight [g] at Day 371 (% control)	Male	13969 (100%)	13484 (97%)	13888 (99%)	14748 (106%)	11575 (83%)	No compound related effect on food consumption was observed. A non-statistically significant trend toward decreased body weight was noted in the high dose group.
	Female	13588[D] (100%)	10412 (77%)	11385 (84%)	10721 (79%)	10382 (76%)	
Ovary abs. wt (g) (% control)	Female	1.940 (100%)	0.889* (46%)	1.217* (63%)	1.034* (53%)	0.789* (41%)	In the absence of statistically significant changes in relative ovary weight and the lack of corresponding histopathological changes, changes in ovary weight are considered unlikely to be treatment-related
Ovary rel. wt (%) ± SD (% control)	Female	0.014 ± 0.001 (100%)	0.009 ± 0.002 (64%)	0.011 ± 0.003 (79%)	0.010 ± 0.005 (71%)	0.008 ± 0.002 (57%)	
Gross Pathology/ Histopathology	Male/Female	-	-	-	-	-	No substance-related gross pathology or histopathology findings were observed.

* = $p \leq 0.05$; D= Premature death of small female from the control group has biased the mean upward in this group; abs. wt = absolute weight; rel. wt = relative weight

Table 43 Detailed findings in study 62

Endpoint	Sex	Concentration [ppm]			
		0	30	125	500
No. animals/group (No. animals/ 4-week recovery group)	Male/Female	15/15 (15/15)	15/15	15/15	15/15 (15/15)
Mortality (recovery group)	Male/Female	0/0 (0/0)	2/1	0/1	1/0 (1/0)
Clinical signs					
Necrosis in head/neck region (maximum incidence)	Male/Female	0/0	0/0	0/0	4/4 (week 2-11)
Uncoordinated gait (maximum incidence)	Male/Female	0/0	0/0	0/0	14/14 (week 1-5)
Poor general condition (maximum incidence)	Male/Female	0/0	0/0	0/0	14/14 (week 1-5)
Haematology					
Erythrocytes (tera/L) after 4 weeks treatment	Male/Female	6.81/7.14	6.89/6.92	7.11/6.94	6.74/6.68**
Erythrocytes (tera/L) after 13 weeks treatment	Male/Female	8.26/7.80	7.99/7.82	8.14/7.81	7.84*/7.48
Haemoglobin (g/L) after 4 weeks treatment	Male/Female	144/146	147/142*	146/140**	133**/138**
Haemoglobin (g/L) after 13 weeks treatment	Male/Female	155/142	149/142	152/148	148/141
Haematocrit (L/L) after 4 weeks treatment	Male/Female	0.453/0.446	0.467/0.439	0.467/0.431**	0.423**/0.427**
Haematocrit (L/L) after 13 weeks treatment	Male/Female	0.472/0.449	0.461/0.435	0.462/0.444	0.456/0.431
Body weight [g]	Male	324	328	317	292**

Endpoint	Sex	Concentration [ppm]			
		0	30	125	500
after 13 weeks treatment main group (% control)		-	(101)	(98)	(90)
	Female	184 -	180 (98)	185 (101)	172 (93)
Body weight [g] after 13 weeks treatment (% control) (after 4 weeks recovery) recovery group	Male	306 - 331 -	-	-	283** (92) 311 (94)
	Female	178 - 193 -	-	-	174 (98) 185* (96)
Liver weight [mg] after 13 weeks treatment	Male, abs. (rel.)	12406 (3774)	11949 (3554*)	11997 (3686)	11299* (3738)
	Male, abs. (rel.) [% control]	100 (100)	96 (94)	97 (98)	91 (99)
	Female, abs. (rel.)	6519 (3441)	6516 (3535)	6570 (3465)	6562 (3708**)
	Female, abs. (rel.) [% control]	100 (100)	100 (103)	101 (101)	101 (108)
Pathology/ Histopathology	Male/Female	-	-	-	-

*=p<0.05; **=p<0.01
abs = absolute weight
rel = liver weight relative to 100 g terminal body weight

Table 44 Detailed findings in study 59

Endpoint	Sex	Concentration (ppm)			
		0	100	300	1000
animals/group (No. animals/ 4-week recovery group)	Male/Female	20/20 (8/8)	20/20 (8/8)	20/20 (8/8)	20/20 (8/8)
Mortality (recovery group)	Male/Female	0/0 (0/0)	0/0 (0/0)	1/0 (0/0)	0/0 (0/0)
Clinical signs observed during 13 week treatment					
Straddle gait	Male	0/20	0/20	0/20	16/20
	Female	0/20	0/20	0/20	15/20
Salivation	Male	0/20	0/20	0/20	5/20
	Female	0/20	0/20	0/20	5/20
Body weight [g] ± SD	Male after 13 weeks	447 ± 32 (100)	442 ± 44 (99)	436 ± 38 (98)	394 ± 40** (88)

Endpoint	Sex	Concentration (ppm)			
		0	100	300	1000
Main study group (% of control) °	treatment				
	Female after 13 weeks treatment	251 ± 22 (100)	254 ± 21 (101)	247 ± 18 (98)	227 ± 24** (90.5)
Body weight [g] ± SD recovery group (% of control) °	Male after 13 weeks treatment	463 ± 43 (100)	450 ± 71 (97)	429 ± 32 (93)	396 ± 24** (86)
	Male after 4 weeks recovery	503 ± 50 (100)	490 ± 75 (97)	466 ± 35 (93)	435 ± 29** (86)
	Female after 13 weeks treatment	248 ± 18 (100)	247 ± 21 (100)	245 ± 15 (99)	230 ± 9* (93)
	Female after 4 weeks recovery	268 ± 23 (100)	251 ± 27 (94)	259 ± 17 (97)	252 ± 18 (94)
Organ weights		-	-	-	-
Pathology/ Histopathology Sciatic nerve, single fibre degeneration after 13 weeks treatment (after 4 weeks recovery)	Male	0	0	0	5 (1)
	Female	0	0	0	3 (0)

*=p<0.05; **=p<0.01
°= no statistical analysis performed

Table 45 Detailed findings in study 63

Endpoint	Sex	Concentration (ppm)			
		0	**10**	**60**	**360**
No. animals/group	Male/Female	4/4	4/4	4/4	4/4
Mortality	Male/Female	0/0	0/0	0/0	0/0
Clinical signs					
Motor disturbance (total occurrence)	Male/Female	0/0	0/0	0/0	3/1 (41x)
Vomiting (total occurrence)	Male/Female	1/0	0/0	0/1 (2x)	1/3 (9x)
Pasty faeces (total occurrence)	Male/Female	0/0	2/0 (2x)	2/0 (2x)	2/0 (5x)
Diarrhoea (total occurrence)	Male/Female	0/1 (2x)	2/0 (3x)	3/1 (5x)	2/3 (14x)
Body weight [kg] (% control)	Male	9.8 (100)	10.5 (107)	9.9 (101)	10.0 (102)

Endpoint	Sex	Concentration (ppm)			
	Female	9.4 (100)	10.0 (106)	9.6 (102)	8.7 (93)
Body weight gain [kg] week 1-13 (% control)	Male	0.9 (100)	1.5 (167)	0.9 (100)	1.1 (122)
	Female	1.0 (100)	1.7 (170)	1.2 (120)	0.4 (40)
Liver weight [g]	Male, abs. (rel.)	368.8 (38.5)	368.0 (35.8)	371.8 (39.2)	374.8 (37.55)
	Female, abs. (rel.)	334.8 (36.05)	336.3 (33.8)	334.0 (35.55)	330.0 (38.3)
Liver weight [%]	Male, abs. (rel.)	100 (100)	100 (93)	101 (102)	102 (98)
	Female, abs. (rel.)	100 (100)	100 (94)	100 (99)	99 (106)
Gross Pathology/ Histopathology	Male/Female	-	-	-	-

Table 46 Detailed findings in study 64

Endpoint	Sex	Concentration (ppm)			
		0	40	160	640
No. animals/group	Male/Female	6/6	6/6	6/6	6/6
Mortality	Male/Female	0/0	0/0	0/0	0/0
Clinical signs					Slight disturbance of movement, especially in the hindlimbs observed in several animals. ↑ vomiting and ↑ diarrhoea
Body weight [kg] (% control)	Male	12.3 (100)	12.8 (104)	13.3 (108)	11.1 (90)
	Female	11.8 (100)	11.6 (98)	11.8 (100)	12.0 (102)
Body weight gain [kg] Week 1-52 (% control)	Male	3.7 (100)	4.2 (114)	4.8 (130)	2.6 (70)
	Female	3.4 (100)	3.4 (100)	3.6 (106)	3.8 (112)
Liver weight [g]	Male, abs. (rel.)	441.7 (36.42)	467.0 (37.25)	461.5 (35.47)	396.3 (36.55)
	Female, abs. (rel.)	421.2 (35.85)	380.7 (32.77)	435.0 (37.07)	431.5 (36.48)
Gross Pathology/ Histopathology	Male/Female	-	-	-	-

abs = absolute body weight
rel = liver weight relative to terminal body weight

4.7.1.2 Repeated dose toxicity: inhalation

Table 47: Summary table of relevant repeated dose inhalation toxicity studies with beta-cyfluthrin

Study	Analyt. conc. [mg/m³ air]	Test substance	NO(A)EC [mg/m³ air]	Targets / Main effects	Reference
5 d, Wistar rat range finding (GLP: yes, OECD 403)	0*-0.25-3.8-28 Aerosol (10 males and 10 females)	Beta-cyfluthrin batch no: 16001/87, purity: 98% Vehicle: ethanol/PEG 400 (1:1)	0.25	≥ 3.8 mg/m³ air : Clinical signs, transient reduction of bw, lung findings	Study 66†
28-day, Wistar rat (GLP: yes, OECD 412)	0-0.2-2.7-23.5 Aerosol (10 males and 10 females/group)	Beta-cyfluthrin batch no: 16001/87, purity:97.9% Vehicle: ethanol/PEG 400 (1:1)	0.2 (0.07 mg/kg bw/d)	≥ 2.7 mg/m³ air : Clinical signs, reduction of bw	Study 67†

* = air and vehicle control
†Key study

Table 48: Summary table of relevant repeated dose inhalation toxicity studies with cyfluthrin

Study	Analyt. conc. [mg/m³ air]	Test substance	NO(A)EC [mg/m³ air]	Targets / Main effects	Reference
90-day, Wistar rat (GLP: no, OECD 413)	0-0-0.09-0.71-4.52 mg/m³ air Aerosol (10 males and 10 females/group)	Cyfluthrin batch no: 816170019, purity: 94.9% Vehicle: polyethylene glycol E 400: ethanol (1 : 1)	0.09 (approx. 0.02 mg/kg bw/d)	≥0.71 mg/m³ air: Behavioural disturbances (agitation, (erected tail), reduction of bw	study 65 †

†Key study

Behavioural disturbances and an effect on body weight gain were noted in inhalation studies with beta-cyfluthrin and cyfluthrin on rats which failed to provide evidence of significant pathological lung changes but resulted in a slight, compensatory acidosis. In the 4-week study with beta-cyfluthrin (study 67, see Table 50), no test substance related findings were apparent in the pathological and histopathological investigations. The slightly changed clinical parameters were interpreted as a result of compensatory reactions due to a slight respiratory acidosis. Additional lung function tests produced no evidence of pathophysiological lung changes. The NOAEC in this study was 0.2 mg beta-cyfluthrin/m³ air (corresponding to approx. 0.07 mg/kg bw/d), based on clinical signs and a reduced body weight gain at the next higher doses.

Table 49 Detailed findings in study 66

Detailed study findings					
Endpoint	Sex	Concentration (mg/m³ air)			
		0	0.25	3.8	28
No. animals/group	Male/Female	10/10	10/10	10/10	10/10
Mortalities	Male/Female	0/0	0/0	0/0	0/0
Clinical signs	Male/Female	0/0	0/0	10/10 piloerection, unpreened hair	10/10 reduced activity, piloerection,

Detailed study findings					
Endpoint	**Sex**	**Concentration (mg/m³ air)**			
		0	0.25	3.8	28
				coat	unpreened hair coat
Pathology/ Histopathology: Hepatoid foci (lung)	Male/Female	0/0	1/0	2/2	3/3*#
Body weight [g] after 4 day treatment (% control)	Male	203 (100)	205 (101)	196 (96)	190** (94)
	Female	190 (100)	187 (98)	185 (97)	178 (94)
Body weight [g] after 21 day treatment (% control)	Male	259 (100)	266 (103)	266 (103)	256 (99)
	Female	200 (100)	197 (99)	201 (101)	197 (99)

*=p<0.05; **=p<0.01
#: statistical analysis was performed over sum male/female

Table 50 Detailed findings in study 67

Detailed study findings						
Endpoint	**Sex**	**Concentration (mg/m³ air)**				**Comment**
		0 (vehicle)	**0.2**	**2.7**	**23.5**	
No. animals/group	Male/Female	10/10	10/10	10/10	10/10	
Mortality	Male/Female	0/0	0/0	0/0	0/0	
Clinical signs	Male/Female	0/0	0/0	0/0	10/10	signs included unkempt fur, piloerection, sometimes a slightly reduced motility but mainly an increased activity
Body and organ weights						
Liver, absolute (mg)	Male/Female	9059/ 7032	8189*/ 6743	8459/ 6506	7844**/ 6747	5885-10607/ 5038-8011#
Liver, relative (mg/100 g bw)	Male/Female	3848/ 3649	3601/ 3574	3833/ 3657	3658/ 3680	No histopathological correlates were observed.

#: 2-sigma ranges of historical control data (lower and upper area)
*=p<0.05; **=p<0.01

Table 51: Detailed findings in study 65

Detailed study findings						
Endpoint	**Sex**	**Concentration mg/m³ air**				**Comment**
		0	0.09	0.71	4.52	
No. animals/group	Male/Female	10/10	10/10	10/10	10/10	
Mortalities	Male/Female	0/0	0/0	0/0	0/0	
Clinical signs						
Non-specific disturbed behaviour	Male	0/10	0/10	0/10	10/10 (Day 13-88)	Agitation and erect tail observed at 4.52 mg/m³ air
	Female	0/10	0/10	10/10 (Day 42-86)	10/10 (Day 9-96)	
Body weight [g] after 12 week treatment	Male	277 (air) 276 (vehicle)	258*#	253**	236**	
	Female	193 (air) 185 (vehicle)	185	178**	182*	
Body weight [% air control] after 12 week treatment	Male	100	93	91	85	
	Female	96	96	92	94	
Histopathology	Male/female					No substance related findings in nerve tissues after histopathology.

*=p<0.05; **=p<0.01; #: statistical analysis compared to air control group data

4.7.1.3 Repeated dose toxicity: dermal

Table 52: Summary table of relevant repeated dose dermal toxicity studies with cyfluthrin*

Study	Dose levels	Test substance	NO(A)EL [mg/kg bw/d]	Targets / Main effects	Reference
3-week dermal toxicity in rabbits (GLP: no, similar to EPA no. 163, 1978)	0, 50, and 250 mg/kg bw/d (New Zealand White) (6 males and 6 females)	Cyfluthrin, batch no.: 16001/79, purity: 83.5% Vehicle: polyethylene glycol 400	250	No effects at any dose level.	Study 68 †
22/23-day dermal toxicity in rats (GLP: yes, OECD 401)	0-100-340-1000 mg/kg bw/d (including recovery at 0 and 1000 mg/kg bw/d) (Sprague-Dawley rats) (8 males and 8 females/ group)	Cyfluthrin, batch no.: 2030025/BF9140-23, purity: 95.5-95.9% No vehicle used (moistened pads)	Systemic: 340 Local: 100	Systemic effects at 1000 mg/kg bw/d: Dark red discharge from the nose in males (including recovery group), urine stains in females, reduced food consumption Local effects (≥ 340 mg/kg bw/d): skin lesions	Study 69 †

* Not-acceptable studies were not included.
†Key study

Studies on dermal toxicity on rat and rabbit are available for cyfluthrin only. In a 3-week study on rabbits no specific effects were observed (study 68, see Table 54).

In a 22/23-day dermal toxicity study in rats (study 69, see Table 53) systemic effects in the form of dark red discharge from the nose and urine staining in males and females, respectively and a reduced food intake occurred at the highest dose of 1000 mg/kg bw/d. From 340 mg/kg bw/d onwards severe skin lesions were noted (ulceration, hyperkeratosis, acanthosis, inflammation, and dermal fibrosis). These changes persisted throughout the recovery period. The systemic NOAEL was established at 340 mg/kg bw/d, the local NOAEL at 100 mg/kg bw/d.

Table 53: Detailed findings in study 69

Detailed study findings						
Endpoint	**Sex**	**Dose**				**Comment**
		0	**100**	**340**	**1000**	
No. animals/group (recovery group)	Male/Female	8/8 (8/8)	8/8	8/8	8/8 (8/8)	
Mortalities	Male/Female	0/0	0/0	0/0	0/0	
Scabs [incidence] (%)	Male	0	0	0	5 (62)	6 (75) in recovery group
	Female	0	1 (12)	6 (75)	6 (75)	6 (75) in recovery group
Treated skin [incidence] (acanthosis, hyperkeratosis, inflammation, ulcer)	Male	-	1#	1	3	Severity: 1-3 (dose dependent)
	Female	-	-	1	7 (6 for ulcer)	Severity: ~3
Body weight [g] after 21 day treatment	Male	310	303.7	303.3	301.1	
	Female	238.6	236.3	232.3	228.3	
Body weight [g] after 14 day recovery	Male	304.7	ND	ND	296.1	
	Female	223.8	ND	ND	217.0	
Food consumption [g/day] after 7 (21) day treatment	Male	24.81 (22.38)	23.15 (21.16)	24.12 (20.99)	20.63* (20.88)	Food consumption comparable to control after 3 weeks and in recovery group at 4 and 5 weeks.
	Female	18.31 (21.3)	17.64 (20.49)	17.27 (21.53)	16.01* (21.26)	
Liver weight [mg], abs.	Male	9893 ± 1358	9544 ± 1010	10224 ± 1226	10727 ± 1874	
	Female	7363 ± 790	7315 ± 995	7240 ± 805	7493 ± 609	
Liver weight [mg], rel.	Male	3.756 ± 0.342	3.680 ± 0.331	3.939 ± 0.421	4.101 ± 0.497	
Liver weight [mg], rel.	Female	3.607 ± 0.206	3.608 ± 0.266	3.718 ± 0.288	3.761 ± 0.295	
Pathology, Histopathology						No test substance related

Detailed study findings						
Endpoint	**Sex**	**Dose**				**Comment**
		0	**100**	**340**	**1000**	
						findings. No findings in nervous system related tissues (brain, optic and sciatic nerve, spinal cord)

#: average severity of effects: 1 (minimal) to 5 (severe)
*=p<0.05; **=p<0.01
abs = absolute body weight
rel = liver weight relative to terminal body weight

Table 54: Detailed findings in study 68

Detailed study findings					
Endpoint	**Sex**	**Dose**			**Comment**
		0	50	250	
Mortalities/ Clinical signs	Male/Female	None	None	None	No substance-related skin findings were observed
Body weight [g] after 3 weeks treatment (% control)	Male, intact skin	2.71 (100)	2.63 (97)	2.77 (102)	No statistical analysis was performed. Groups were considered comparable.
	Female, intact skin	3.14 (100)	3.00 (96)	3.03 (96)	
	Male, abraded skin	2.92 (100)	2.66 (91)	2.86 (98)	
	Female, abraded skin	3.09 (100)	3.00 (97)	3.03 (98)	
Organ weights; haematology, clinical chemistry, pathology/histopathology	Male/Female	-	No findings observed, no deviations from control	No findings observed, no deviations from control	
Liver weight [mg] (% control)	Male, intact skin	90535 (100)	88351 (98)	107259 (118)	
	Female, intact skin	79277 (100)	74849 (94)	86458 (109)	

4.7.1.4 Repeated dose toxicity: other routes

No other routes were tested.

4.7.1.5 Human information

No human information exists for repeat-dose exposure of beta-cyfluthrin.

4.7.1.6 Summary and discussion of repeated dose toxicity

Oral:

In short-term toxicity experiments in rats and dogs oral administration of beta-cyfluthrin or cyfluthrin led to similar adverse effects: mortality, general behavioural disturbances, motor disturbances, lower body weight development, choreoathetotic signs, vomiting and diarrhea. No relevant effects on haematological, clinico-chemical and urine analytical parameters were detected.

The lowest NOAEL of 1 mg/kg bw/d was derived from a 4-week study with beta-cyfluthrin (study 61). At the next higher dose of 4 mg/kg bw/d mortality, clinical signs, reduced body weight development and an increased liver weight were noted.

In a 90-day oral study in rats with beta- cyfluthrin (study 62) mortality, clinical signs, reduced body weight and skin lesions was noted at approx. 37 mg/kg bw/d. The NOAEL was 9.5 mg/kg bw/d.

In a 90-day oral study in rats with cyfluthrin gait abnormalities, salivation and a reversible slight axonal degeneration was reported in some rats dosed with 300 and 1000 ppm (19 and 60.9 mg/kg bw/d) (study 59). The NOAEL was 6.2 mg/kg bw/d.

In a 90-day study on dogs with beta-cyfluthrin (study 63) the NOAEL of 60 ppm, equal to 2.4 mg/kg bw/d, was based on motor disturbances, vomiting, diarrhea and a reduced body weight gain in females at the next higher dose of 14 mg/kg bw/d.

A 12-month feeding study in dogs with cyfluthrin (study 60) revealed slight to severe motor disturbances, vomiting, diarrhea and a reduction in body weight gain at ≥360 ppm (10.6-18 mg/kg bw/d). The study revealed a NOAEL of 100 ppm (2.4/3.6 mg/kg bw/d). This study supersedes the 12-month feeding study in dogs (study 64) which was considered not acceptable.

Inhalation:

Behavioural disturbances and an effect on body weight gain were noted in 5-day and 28-day inhalation studies on rats with beta-cyfluthrin which failed to afford evidence of significant pathological lung changes but resulted in a slight, compensatory acidosis. In the 28-day study (study 67), no test substance related findings were apparent in the pathological and histopathological investigations. The slightly changed clinical parameters were interpreted as a result of compensatory reactions due to a slight respiratory acidosis. Additional lung function tests produced no evidence of pathophysiological lung changes. The NOAEC in this study was 0.2 mg beta-cyfluthrin/m³ air (corresponding to approx. 0.07 mg/kg bw/d).

Dermal:

Studies on dermal toxicity on rat and rabbit are available for cyfluthrin only. In a 3-week study on rabbits with cyfluthrin no specific effects were observed (study 68, see Table 54).

In a 22/23 day-dermal toxicity study in rats (study 69, see Table 53) systemic effects in the form of dark red discharge from the nose and urine staining in males and females, respectively, and a reduced food intake occurred at the highest dose of 1000 mg/kg bw/d. From 340 mg/kg bw/d onwards severe skin lesions were noted (ulceration, hyperkeratosis, acanthosis, inflammation, and dermal fibrosis). These changes persisted throughout the recovery period. The systemic NOAEL was established at 340 mg/kg bw/d, the local NOAEL at 100 mg/kg bw/d.

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

Table 55: Results of repeat-dose toxicity studies in comparison with CLP criteria

Study type	STOT RE 1	STOT RE 2	Toxicological result (NOAEL/NOAEC and LOAEL/LOAEC)	Significant/severe effects at LOAEL
28-day oral rat	≤ 30mg/kg bw/d	≤ 300mg/kg bw/d	NOAEL: 1 mg/kg bw/d LOAEL: 4 mg/kg bw/d	Mortality, clinical signs, reduced body weight development, increased liver weight
90-day, oral, rat	≤ 10 mg/kg bw/d	≤ 100 mg/kg bw/d	NOAEL: 9.5 mg/kg bw/d LOAEL: 38.9 mg/kg bw/d (males); 42.4 mg/kg bw/d (females)	Mortality, clinical signs, reduced body weight and water intake, skin lesions, reduced red blood cell parameters, increased calcium levels in urine
			NOAEL: 6.2 mg/kg bw/d LOAEL: 18.98 mg/kg bw (males); 21.22 mg/kg bw (females)	Gait abnormalities, salivation, slight axonal degeneration of sciatic nerve (reversible)
90-day, oral, dog	-	-	NOAEL: 2.4 mg/kg bw/d LOAEL: 14 mg/kg bw/d	Motor disturbances (hind limb), vomiting, diarrhea, reduced body weight
12-month, oral, dog	-	-	NOAEL: 2.4 mg/kg bw/d (males); 3.6 mg/kg bw (females) LOAEL: 10.64 mg/kg bw (males); 10.74 mg/kg bw (females)	Reduced body weight, neurological disorders (gait abnormalities)
5-day, inhalation, rat	-	-	NOAEC: 0.25 mg/m³ air LOAEC: 3.8 mg/m³ air	Clinical signs, transient reduction of body weight, lung findings
28-day, inhalation, rat	≤ 0.6 mg/litre/6h/day	≤ 3 mg/litre/6h/day	NOAEC: 0.07 mg/kg bw/d LOAEC: 0.94 mg/kg bw/d	Clinical signs, reduction of body weight
90-day, inhalation, rat	≤ 0.2 mg/litre/6h/day	≤ 1 mg/litre/6h/day	NOAEC: 0.02 mg/kg bw/d LOAEC: 0.16 mg/kg bw/d	Behavioural disturbances (agitation, (erected tail), reduction of body weight
28-day, dermal, rabbit	≤ 60 mg/kg bw/d	≤ 600 mg/kg bw/d	21-day, dermal, rabbit: NOAEL: 250 mg/kg bw/d	No effects at any dose level
28-day, dermal, rat	≤ 60 mg/kg bw/d	≤ 600 mg/kg bw/d	22/23-day, dermal, rat: NOAEL: 340 mg/kg bw/d LOAEL: 1000 mg/kg bw/d	Dark red discharge from the nose in males (including recovery group), urine stains in females, reduced food consumption

4.7.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

Even though some of the observed findings were severe (such as clinical signs, motor disturbances and/or gait abnormalities), they were considered to represent acute toxic/neurotoxic effects of beta-cyfluthrin/cyfluthrin. Due to intensive metabolism and rapid excretion of beta-cyfluthrin/cyfluthrin (see Chapter 4.1 ADME studies), daily administrations of beta-cyfluthrin/cyfluthrin are considered to represent a sequence of acute intoxications. A proposal for classification for acute effects is already made. Hence, it is proposed not to classify beta-cyfluthrin/cyfluthrin for STOT-RE "Danger of serious damage to health by prolonged exposure".

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

Repeat dose toxicity studies with cyfluthrin or beta-cyfluthrin are available for the rat (dietary, gavage, inhalation and dermal exposure), dog (dietary exposure), mouse (dietary exposure) and rabbit (dermal exposure).

Mortality and clinical signs of neurotoxicity (e.g. abnormal gait) were observed below the guidance values for classification in several studies. However, these effects were considered to represent acute toxic/neurotoxic effects, already covered by the proposed classification with Acute Tox. 2. Due to intensive metabolism and rapid excretion of cyfluthrin and beta-cyfluthrin, daily administrations of the substances were considered to represent a sequence of acute intoxications. Therefore, the DS proposed no classification for STOT RE.

Comments received during public consultation

Comments on the STOT RE classification of cyfluthrin and/or beta-cyfluthrin were received from 2 MSCAs. One MSCA supported the DS's proposal of no classification while the other one proposed classification with STOT RE 2 (nervous system), pointing out that effects occurred significantly below the LD_{50} values in some repeat dose studies. They also mentioned histopathological findings in the nervous system in study 59. In their response the DS reiterated that the clinical signs were acute effects addressed by the proposed acute toxicity and STOT SE 3 classifications.

Assessment and comparison with the classification criteria

No other effects potentially relevant for a STOT RE classification apart from neurotoxicity were observed in the available studies.

Clinical signs of neurotoxicity such as gait abnormalities were observed in many single dose and repeat dose studies with cyfluthrin and beta-cyfluthrin. Based on the information available to RAC, the neurotoxic effects in the repeat dose studies seem to represent a series of acute intoxications. For example, in a 90-d rat dietary study with cyfluthrin (study

59), straddle gait appeared in 13 out of 40 animals on day 1, in 25 animals on day 3 and the incidence started to decrease from week 4 at a dose of 61/68 mg/kg bw/d (m/f).

Slight axonal degeneration of single nerve fibres in the sciatic nerve was observed in 8 out of 40 animals in a 90-d rat dietary study with cyfluthrin (study 59) at 61/68 mg/kg bw/d (m/f), which could potentially support a STOT RE classification. However, minimal single fibre degeneration in the sciatic nerve was observed in 6 out of 8 rats (vs. none in controls) already after a single gavage dose of 80 mg/kg bw cyfluthrin in PEG 400 in another study (Anonymous, 1983). Thus, the histopathological findings in study 59 do not necessarily represent a repeat dose effect.

Taking into account the temporal pattern of the neurotoxic findings, classification for acute toxicity and STOT SE is considered more appropriate than a STOT RE classification. **RAC agrees with the DS that no classification is warranted for STOT RE**.

4.8 Germ cell mutagenicity (Mutagenicity)

Hazard class not assessed in this dossier.

4.9 Carcinogenicity

Hazard class not assessed in this dossier.

4.10 Toxicity for reproduction

4.10.1 Effects on fertility

4.10.1.1 Non-human information

Fertility studies were conducted with cyfluthrin only (Table 56).

Table 56: Summary table of relevant reproductive toxicity studies*

Study	Dose levels	Test substance	NO(A)EL	Targets / Main effects	Reference
2-gen. study OECD 416 Oral, diet, SD rat GLP: yes	0-50-125-400 ppm (3-7, 9-19, 29-59 mg/kg bw/day) Default calculation for males and females: 0-3.3-8.3-26.7 mg/kg bw/d (30 males and 30 females/ group)	Cyfluthrin, batch no. 2030025, purity 94.6-96.2%	NOAEL parental: 50 ppm (3.3 mg/kg bw/d) NOAEL offspring: 50 ppm (3.3 mg/kg bw/d) NOAEL reproductive: 400 ppm (26.7 mg/kg bw/d)	Parental: ≥125 ppm: Splaying of the hind limbs in females; ≥ 400ppm: decreased bw Offspring: ≥125 ppm: Coarse tremors, decreased bw	Study 70 †
Supplemental 2-gen study OECD 416 Oral, diet; SD rat	0-25-50 ppm (1.9-4.1, 3.8-8.0 mg/kg bw/d) Default calculation for males and	Cyfluthrin, batch no. 2030025, purity 94.6-96.2%	NOAEL reproductive, offspring, parental: 50 ppm (3.3 mg/kg bw/d)	No effects	Study 71

Study	Dose levels	Test substance	NO(A)EL	Targets / Main effects	Reference
(supplemental) GLP: yes	females: 0-1.7-3.3 mg/kg bw/d (30 males and 30 females/ group)				

* Not-acceptable studies were not included. For further study details, please see also IUCLID ECHA DocIII cyfluthrin.
†Key study

In a 2-generation study in Sprague-Dawley rats (study 70), the F_0 and F_1 adults received cyfluthrin in the diet throughout the entire study, beginning at seven weeks of age for the F_0 adults and at weaning for the F_1 adults. Prior to breeding, the animals received treated feed at least for a ten-week period. The following dose levels were administered female parental animals (for risk assessment purposes, a time-weighted conversion factor of 15 was used for calculation of the test substance intake based on the test substance feed concentration, as proposed by the WHO (2009): 50 ppm (default 3.3 mg/kg bw/d), 125 ppm (default 8.3 mg/kg bw/d), 400 ppm (default 26.7 mg/kg bw/d). During the study, adult animals were evaluated for the effect of the test compound on body weight, food consumption, clinical signs, oestrus cycling, mating, fertility, gestation length, and litter size. The offspring were evaluated for compound-related effects on sex ratio, pup viability, body weight gain, and clinical signs. Gross necropsy evaluations were performed on all adults and pups. Histopathologic evaluation of reproductive organs, the pituitary, and gross lesions was performed on all F_0 and F_1 adults. Additionally due to clinical signs of neurotoxicity, the brain, spinal cord, and one sciatic nerve were collected from all F1 adults and placed in buffered 10 percent formalin in the event that further microscopic examination was deemed necessary.

There were no compound-related clinical signs for adult males. In F_0 and F_1 females, a compound-related and statistically significant increased incidence of splayed hind limbs occurred at 400 ppm during the lactation phase (Table 57).

Table 57: Rat 2-gen. study: Incidence of splayed hind limbs in females during lactation (study 70)

Generation	Incidence of splayed hind limbs in dose group females during lactation			
	0 ppm	50 ppm	125 ppm	400 ppm
F0 females	(0 / 30)	(0 / 27)	(0 / 26)	(15 / 29)** (52%)
F1 females	(0 / 25)	(0 / 27)	(0 / 27)	(9 / 25)** (36%)

Statistically significant (Fisher's Exact Test): * = $p \leq 0.05$; ** = $p \leq 0.01$

There were no compound-related mortalities in parental animals. Statistically significantly decreased terminal body weights were observed in F1 males at 125 ppm and 400 ppm and in F1 females at 400 ppm. There were no compound-related absolute or relative organ weight changes in the F_0 and F_1 adults. During the lactation period decreases in food consumption were observed at 400 ppm in both the F_0 and F_1 females (values ranged from 78% - 85% on the F_0 females and from 77% - 88% during days 0-21 post partum in the F_1 females compared to the control and lower dose groups). There were no effects on adult reproductive parameters (oestrus cycle staging; insemination length; mating, fertility and gestation indices; gestation length; number of implantation sites and birth index. No compound-related gross and histopathological lesions were observed.

Coarse tremors were observed in the F_1 and F_2 pups at and above 125 ppm (Table 58). The tremors were observed as early as lactation day 5 and had ceased by lactation day 18. The increased incidence of coarse tremors and the decreased pup body weight in F_1 and F_2 pups at and above 125 ppm (19

and 59 mg/kg bw/d) occurred in the presence of maternal toxicity (splayed hind limbs, severity not indicated).

The excretion and concentration of cyfluthrin in rat milk has not been determined but it can be concluded that the presence of adverse effects in the offspring at 125 ppm was due to transfer of cyfluthrin or of its metabolite(s) in the milk during the lactation period. This conclusion is supported by the absence of adverse treatment effects on prenatal or peri-natal litter parameters.

Table 58: Rat 2-gen. study: Litter incidence of coarse tremors (study 70)

Generation	Litter incidence of coarse tremors in pups observed during lactation			
	0 ppm	50 ppm	125 ppm	400 ppm
F1 pups	(0 / 30)	(0 / 27)	(4 / 25)	(15 / 28)*
F2 pups	(0 / 25)	(0 / 26)	(19 / 26)*	(9 / 25)*

Statistics: Chi-square test & Fisher's Exact test (Bonferroni adjustment of the p value)

In addition, at 400 ppm, clinical signs of neurotoxicity (splayed hind limbs) were observed in F0 and F1 females during lactation.

There was no substance-related effect on pup gender, litter size; live birth, viability and lactation indices or gross lesions in the F_1 or F_2 pups. Cyfluthrin administration to F_0 and F_1 parents had no effect on birth weight of their offspring.

The parental and offspring NOAEL is 50 ppm, equivalent to 3.3 mg/kg bw/day (default calculation for males and females). Fertility parameters were not affected by cyfluthrin at doses up to and including 400 ppm (equivalent to 26.7 mg/kg bw/day).

The NOAEL of 50 ppm (3.3 mg/kg bw/d) was confirmed in a supplemental 2-generation study (study 71) showing that transient reductions in pup weight noted in the previous study at 50 ppm were not test-substance related.

4.10.1.2 Human information

No data available.

4.10.2 Developmental toxicity

4.10.2.1 Non-human information

Teratogenicity studies with oral administration were conducted in rats and rabbits with cyfluthrin and beta-cyfluthrin.

Table 59: Summary table of relevant oral teratogenicity studies with beta-cyfluthrin*

Study	Dose levels	Test substance	NO(A)E(C)L	Targets / Main effects	Reference
Teratogenicity; Wistar rats Gavage 6th -15th day of gestation (GLP: yes, OECD 414)	0–3–10–40 mg/kg bw/d (20 females/ group)	beta-cyfluthrin technical, batch-no.: 3030125,purity: 96.5-97.3% vehicle: 1% aqueous Cremophor	NOAEL maternal: 3 mg/kg bw/d NOAEL developmental: 10 mg/kg bw/d	Maternal: 40 mg/kg bw/d: Mortality, clinical findings (hypoactivity, locomotor incoordination, salivation); ≥10 mg/kg bw/d: decreased body	study 76 †

Study	Dose levels	Test substance	NO(A)E(C)L	Targets / Main effects	Reference
				weight gain and food consumption Offspring: 40 mg/kg bw/d: decreased weight; retarded ossification	

* Not-acceptable studies were not included. For further study details, please see also IUCLID ECHA Vol. 3beta-cyfluthrin.
†Key study

Table 60: Summary table of relevant oral teratogenicity studies with cyfluthrin*

Study	Dose levels	Test substance	NO(A)E(C)L	Targets / Main effects	Reference
Teratogenicity; BAY:FB 30 rats Gavage 6th -15th day (GLP: no, OECD 414)	0-3-10-30 mg/kg bw/d (25 females/ group)	cyfluthrin batch no: 16001/79, purity: approx. 85% vehicle: polyethylene glycol E 400	NOAEL maternal: 3 mg/kg bw/d NOAEL developmental: 30 mg/kg bw/d	Maternal: ≥10 mg/kg bw/d: High-stepping gait, ataxia, reduced motility Offspring: No effects	Study 72†
Teratogenicity; Wistar rats Gavage 6th -15th day of gestation (GLP: yes, OECD 414)	0-1-3-10 mg/kg bw/d (25 females/ group)	cyfluthrin (batch no: 816170019,purity 93.4%) vehicle: cremophor EL/distilled water (1% v/v)	NOAEL maternal and developmental: ≥10 mg/kg bw/d	No effects	Study 73
Teratogenicity; Himalayan rabbits, gavage, 6th -18th day of gestation (GLP: no, OECD 414)	0-5-15-45 mg/kg bw/d (15 females/ group)	cyfluthrin (batch no. 816170019, purity: 95.0%) vehicle: Cremophor EL/water (0.5%)	Maternal: 15 mg/kg bw/d Developmental: 45 mg/kg bw/d	Maternal: ≥ 45 mg/kg bw/d: Abortion Offspring: No effects	Study 74 †
Teratogenicity; Chinchilla rabbits, gavage, 6th -18th day of gestation (GLP: yes, OECD 414)	0-20-60-180 mg/kg bw/d (16 females/ group)	cyfluthrin (batch no.: 2380051769,purity 96.0%) formulated in corn oil	Maternal: 20 mg/kg bw/d Developmental: 20 mg/kg bw/d	Maternal: ≥60 mg/kg bw/d: decreased food consumption, bw loss Offspring: ≥60 mg/kg bw/d: Increased post-implantative resorptions	Study 75†

* Not-acceptable studies were not included. For further study details, please see also IUCLID ECHA DocIIIA cyfluthrin
†Key study

In rats, a maternal NOAEL of 3 mg/kg bw/d was derived in the teratogenicity study 72with cyfluthrin (see Table 61). A high-stepping gait, occasionally ataxia and reduced motility were observed in a few dams after administration of the mid- and high-dose (10 and 30 mg/kg bw/d). Doses up to 30 mg/kg bw had no lethal effect and did not affect average weight gain. No general, embryotoxic and/or teratogenic effects were observed in the offspring, resulting in a developmental NOAEL of 30 mg/kg bw/d.

The maternal NOAEL of 3 mg/kg bw/d was confirmed in an oral teratogenicity study 76 with beta-cyfluthrin. An increased incidence of mortality and clinical findings (hypoactivity, locomotor incoordination, salivation) were confined to the high-dose group (40 mg/kg bw/d). From 10 mg/kg bw/d onwards a reduction in body weight gain was noted in the dams. A decrease in foetal weight gain and a retarded ossification was noted at 40 mg/kg bw/d and a developmental NOAEL of 10 mg/kg bw/d was derived.

Likewise, no effects were noted in the offspring of Himalayan rabbits up to oral doses of 45 mg cyfluthrin/kg bw/d. The maternal NOAEL was 15 mg/kg bw/d, based on abortion (study 74, see Table 63).

In Chinchilla rabbits (study 75, see

Table 64, Table 65, Table 66 and Table 67) the maternal and developmental NOAEL was 20 mg cyfluthrin /kg bw/d based on decreased food consumption and body weights loss in the dams and on an increased incidence of post-implantative resorptions in the offspring.

Table 61: Detailed findings in study 72

Detailed study findings in maternal rats						
Endpoint	**Sex**	**Dose mg/kg bw/day**				**Comment**
		0	**3**	**10**	**30**	
No. animals/group (inseminated rats)	Female	25	25	25	25	
Mortality	Female	0	0	0	0	
Clinical signs						Clinical signs were considered to be treatment-related
High stepping gait°	Female	0	0	6	6	Findings were observed occasionally from 2nd week of application
Ataxia°	Female	No	No	Yes #	Yes #	#: observed occasionally in individual animals (no numbers available)
Reduced motility°	Female	No	No	Yes #	Yes #	#: observed occasionally in individual animals (no numbers available)

°= no statistical analysis performed

Table 62: Detailed findings in study 73

Detailed study findings in maternal rats						
Endpoint	**Sex**	**Dose mg/kg bw/day**				**Comment**
		0	**1**	**3**	**10**	
No. animals/group (inseminated rats)	Female	25	25	25	25	
Mortality	Female	0	0	0	0	
Clinical signs (overall incidence)	Female	0	0	2	0	Clinical signs were considered to be not treatment-related, as they occurred in isolated animals and were not observed at

Detailed study findings in maternal rats						
Endpoint	**Sex**	**Dose mg/kg bw/day**				**Comment**
		0	**1**	**3**	**10**	
						the highest dose tested
Partial loss of hair (from day 8 after mating)°	Female	0	0	1	0	Female No. 62 was affected
Colporrhagia (on day 18 after mating)°	Female	0	0	1	0	Female No. 63 was affected

°= no statistical analysis performed

Table 63: Detailed findings in study 74

Detailed study findings: dams						
Endpoint	**Sex**	**Dose mg/kg bw/day**				**Comment**
		0	**5**	**15**	**45**	
No. animals mated/ group	♀	15	15	15	15	
No. animals fertilised / group	♀	15	15	13	14	
No. animals pregnant at termination/ group (%)	♀	15 (100)	15 (100)	13 (100)	11 (78.5)	At 45mg/kg bw/day two dams aborted on days 25 and 28 p.c. and one dam completely resorbed her implants.
Abortions [incidence]	♀	0	0	0	3	
Mortality [incidence]	♀	0	0	0	0	
Bodyweight gain (g) – dosing period [mean/ group]	♀	78.7	57.7	109.6	81.4	
Placenta weight (g) [mean/ group]	♀	4.27	4.26	4.20	4.60	
No. implantation sites [mean/ group]	♀	7.3	6.3	8.5*	6.8	
No. pre-natal losses [mean/ group]	♀	0.6	0.7	1.4	1.8	
Detailed study findings: fetuses following caesarean section day 29 p.c.						
No. foetuses [total/group]	Both sexes	100	84	92	70	
No. foetuses [mean/ group]	Both sexes	6.7	5.6	7.1	5.0	
No. foetuses [mean/ sex/ group]	Male/female	3.3/3.4	2.7/2.9	3.8/3.3	2.8/2.2	

Detailed study findings: dams						
Endpoint	**Sex**	**Dose mg/kg bw/day**				**Comment**
		0	**5**	**15**	**45**	
No. small foetuses/ group (<25g)	Both sexes	1	4	0	0	
Fetal weight (g) [mean/ group]	Both sexes	37.37	37.00	38.77	40.30	
Ossification changes [total foetuses/group] (%)	Both sexes	0 (0)	2 (2.4)	0 (0)	0 (0)	
Ossification changes [total litters/group] (%)	Both sexes	0 (0)	2 (13)	0 (0)	0 (0)	
Malformations						
Arthrogryposis [total foetuses/group] (%)	Both sexes	0 (0)	2 (2.4)	2 (2.2)	3 (4.3)	
Arthrogryposis [total litters/group] (%)	Both sexes	0 (0)	1 (7)	2 (15)	1 (9)	
Tail vertebrae located asymmetrically and adherent [total foetuses/group] (%)	Both sexes	0 (0)	0 (0)	4 (4.3)	0 (0)	
Tail vertebrae located asymmetrically and adherent [total litters/group] (%)	Both sexes	0 (0)	0 (0)	1 (7.7)	0 (0)	

*=p<0.05; **=p<0.01

Table 64: Detailed findings in study 75, parental data

Dose: [mg/kg bw]	**0**	**20**	**60**	**180**
Group size (pregnant animals)	16	13	16	15
Food intake 6-11 p.c. (g/animal/day) [mean/ group]	146	124	107*	76**
Food intake 24-28 p.c. (g/animal/day) [mean/ group]	121	146	161**	178**
Body weight gain [g] (6-19 d) [mean/ group]	-40	-34	-189**	-233**
Body weight gain [g] (6-28 d) [mean/ group]	87	143	42	-6
Gravid uterus weight [g] [mean/ group]	508	450	455	464

*=p<0.05; **=p<0.01

Table 65: Detailed findings in study 75, reproduction data

Dose: [mg/kg bw]	**0**	**20**	**60**	**180**
Number of pregnant dams/ group	16	13	16	15
Implantation sites (% of corpora lutea) [mean/ group]	193 (96.0)	128 (90.8*)	183 (94.3)	186 (98.4)
Pre-implantation loss [No.] (% of corpora lutea) [mean/ group]	8 (4)	13 (9.2*)	11 (5.7)	3 (1.6)
Post-implantation loss [No.] (% of implantation sites) [mean/ group]	21 (10.9)	14 (10.9)	36 (19.7*)	53 (28.5**)
Post-implantation loss, dams affected [No.] (%) [per group]	11 (69)	7 (54)	13 (81)	12 (80)
Embryonic/fetal deaths, total (% of implantation sites) [mean/ group]	21 (10.9)	14 (10.9)	36 (19.7*)	47 (25.3**)
Embryonic resorptions, total (% of implantation sites) [mean/ group]	7 (3.6)	8 (6.3)	21 (11.5**)	28 (15.1**)
Embryonic	5	4	10	10

Dose: [mg/kg bw]	0	20	60	180
resorptions, dams affected [No.] (%) [per group]	(31)	(31)	(63)	(67)
Fetal resorptions, total (% of implantation sites) [mean/ group]	14 (7.3)	6 (4.7)	15 (8.2)	19 (10.2)
Fetal resorptions, dams affected [No.] (%) [per group]	9 (56)	5 (38)	7 (44)	8 (53)
Total fetuses [No.] (% of implant. sites) [per group]	172 (89.1)	114 (89.1)	147 (80.3*)	133 (71.5**)
Total fetuses [No.] [mean/ dam]	10.8	8.8	9.2	8.9
Live fetuses [No.] [per group]	172	114	147	133
Abnormal foetuses [No.] (% of foetuses) [per group]	4 (2.3)	1 (0.9)	3 (2.0)	3 (2.3)
Abnormal foetuses, dams affected [No.] (%) [per group]	4 (25)	1 (8)	3 (19)	3 (20)
Sex of fetuses: male / female (% male) [mean/ group]	86/86 (50)	71/43 (62.3*)	80/67 (54.4)	72/61 (54.1)
Fetal weight (g): male / female, individual basis (male / female, litter basis) [mean/ group]	29/28 (30/29)	31/32** (31/33)	30/29 (31/30)	30/31* (31/32)

*=p<0.05; **=p<0.01

Table 66: Abnormal findings in study 75

Dose: [mg/kg bw]	0	20	60	180
Number of foetuses examined	172	114	147	133
Number of litters examined	16	13	16	15
Type of abnormal finding [No./ group] - External and visceral examination data				
Omphalocele	1	1	0	0
Arthrogryposis	1	0	0	0
Open eye	2	0	0	0
Runt	2	0	3	3
Cheilognathopalatotschisis	1	0	0	0
Cranioschisis	1	0	0	0

Dose: [mg/kg bw]	0	20	60	180
Hemidiaphragm	0	0	0	1
Head	0	0	0	0

Table 67: Skeletal examination data in study 75

Dose: [mg/kg bw]	0	20	60	180
Number of foetuses examined	172	114	147	133
Number of litters examined	16	13	16	15
Type of abnormal finding [No.] - Skeletal examination data				
Total No. of abnormal findings in fetuses (litters) / group	3 (3)	3 (3)	4 (2)	5 (4)
Abnormally Ossified and Fused Sternebrae, fetal data[No.] (%) [per group]	1 (0.6)	0 (0)	1 (0.7)	2 (1.5)
Abnormally Ossified and Fused Sternebrae, litter data [No.] (%) [per group]	1 (6.3)	0 (0)	1 (6.3)	2 (13.3)
Thoracic/Lumbar Vertebral Bodies/Arches Fused, Missing or Bipartite, fetal data [No.] (%) [per group]	1 (0.6)	2 (1.8)	0 (0)	2 (1.5)
Thoracic/Lumbar Vertebral Bodies/Arches Fused, Missing or Bipartite, litter data [No.] (%) [per group]	1 (6.3)	2 (15.4)	0 (0)	2 (13.3)
Ribs fused, bifurcated or missing, fetal data [No.] (%) [per group]	0 (0)	2 (1.8)	2 (1.4)	3 (2.3)
Ribs fused, bifurcated or missing, litter data [No.] (%) [per group]	0 (0)	2 (15.4)	2 (12.5)	2 (13.3)
Partial aplasia of the cranium (Os nasale,	1 (0.6)	0 (0)	0 (0)	0 (0)

Dose: [mg/kg bw]	0	20	60	180
frontale, parietale), fetal data [No.] (%) [per group]				
Partial aplasia of the cranium (Os nasale, frontale, parietale), litter data [No.] (%) [per group]	1 (6.3)	0 (0)	0 (0)	0 (0)
Abnormal Structure of the Vertebral Column and Ribs; Scoliosis, Shortened Trunk, Fused, Bipartite or Missing Vertebral Bodies and Arches; Bifurcated or Missing Ribs, fetal data [No.] (%) [per group]	0 (0)	1 (0.9)	0 (0)	0 (0)
Abnormal Structure of the Vertebral Column and Ribs; Scoliosis, Shortened Trunk, Fused, Bipartite or Missing Vertebral Bodies and Arches; Bifurcated or Missing Ribs, litter data [No.] (%) [per group]	0 (0)	1 (7.7)	0 (0)	0 (0)
Os nasale distally incompletely ossified, fetal data [No.] (%) [per group]	0 (0)	0 (0)	1 (0.7)	0 (0)
Os nasale distally incompletely ossified, litter data [No.] (%) [per group]	0 (0)	0 (0)	1 (6.3)	0 (0)
Tip of the tail missing, fetal data [No.] (%) [per group]	0 (0)	0 (0)	0 (0)	1 (0.8)
Tip of the tail missing, litter data [No.] (%) [per group]	0 (0)	0 (0)	0 (0)	1 (6.7)

*=p<0.05; **=p<0.01

Table 68: Summary table of relevant inhalation teratogenicity studies with cyfluthrin*

Study	Dose levels	Test substance	NO(A)E(C)L	Targets / Main effects	Reference
Teratogenicity; Wistar rats, aerosol, head-nose exposure 6th -15th day of gestation, 6 h per day (GLP: yes, OECD 414)	1st exp.: 0-1.1-4.7-23.7 mg/m³ air 2nd exp.: 0-0.09-0.25-0.59-4.16 + O_2 mg/m³ air (30 females/group)	cyfluthrin (1st exp. batch no.: 233490583, purity: 92.9-93%; 2nd exp. batch no.: 238005176, purity 96.2%) formulated in ethanol/ polyethylene glycol E 400 as aerosol	Maternal and developmental: 0.59 mg/m³ air	≥1.1 mg/m³ air: reduced bw development, reduced fetal weight, retarded ossification In addition ≥ 4.16 mg/m³ air+O_2: Clinical signs of the dams In addition at 23.7 mg/m³ air: Increased incidence of resorptions increased frequency of microphthalmia	Study 77 †
Teratogenicity; Wistar rats, aerosol, head-nose exposure 6th -15th day of gestation, 6 h per day (GLP: yes, OECD 414)	0-0.46-2.55-11.9-12.8+O_2 mg/m³ air (25 females/group)	cyfluthrin (batch no.: 238005176 , purity 94.7-96.2%) formulated in ethanol/ polyethylene glycol E 400	Maternal: <0.46 mg/m³ air Developmental: 0.46 mg/m³ air	≥0.46 mg/m³ air: Decreased food intake and bw development in dams, hypothermia and bradypnoea (hypoventilation) in dams In addition ≥2.55 mg/m³ air: Clinical signs in dams, retarded development of fetuses In addition ≥11.9 mg/m³ air: Respiratory disturbances and hypoactivity in dams, higher incidence of microphthalmia and anophthalmia	Stuy 78 †
Determination of the FCR 1272 concentration in the plasma of rats following inhalation exposure (GLP: no, guideline: not applicable)	0.5, 2.5, 12.5 and 12.5 + O_2 mg/m³ air (5 pregnant females/group)	cyfluthrin (batch no.: 380267024,purity 92%)first dissolved in 5 mL 1,4-dioxane, this solution made up to 50 mL with n-hexane	Not applicable	Very low concentrations of cyfluthrin were only found in the high-dose groups 12.5 mg/m³ air and 12.5 mg/m³ air (+39% oxygen).	Study 79

* Not-acceptable studies were not included. For further study details, please see also IUCLID ECHA DocIIIA cyfluthrin.
†Key study

Inhalation exposure to cyfluthrin caused a physiological maternal compensation mechanism (hypothermia with respiratory alkalosis) followed by reflex bradypnoea after sensory irritation (Table 68).

In the dams food intake of dams were decreased and body weight development was delayed at levels of 0.46 mg/m³ air and above (Table 70).
Clinical signs (bloody snout, ruffled fur) were apparent in the dams at 2.55 mg/m³ air and above. Respiratory disturbances and hypoactivity were noted at 11.9 mg/m³ air and 12.8 mg/m³ air (plus oxygen), and a high-stepping gait at 11.9 mg/m³ air only.

Placental weights were lower from 2.55 mg/m³ air onwards and fetuses showed signs of retarded development (reduction of fetal weight) (Table 69 and Table 70).
No gross pathological findings were recorded at necropsy of any dose group (including the satellite groups).

A NOAEL of < 0.46 mg/m³ air resulted for maternal toxicity, based on decreased food intake and body weight development in dams at this dose.

Table 69: Selected symptoms and clinical observations in dams (study 78)

Dose [mg/m³ air]	**0 a.**	**0 v.**	**0.46**	**2.55**	**11.9**	**O2+12.8**
Number of dams per dose group	25	25	25	25	25	25
Ruffled fur	0	0	0	1 (4%)	19 (76%)	21 (84%)
Retarded breathing	0	0	0	0	17 (68%)	10 (40%)
Laboured breathing	0	0	0	0	5 (20%)	0
Hypoactivity	0	0	0	0	5 (20%)	1 (4%)
High stepping gait	0	0	0	0	5 (20%)	0
Bloody snout	0	0	0	1 (4%)	2 (8%)	2 (8%)

a = air control, v = vehicle control

Table 70: General examination and reproduction data (study 78)

Dose [mg/m³ air]	**0 a.**	**0 v.**	**0.46**	**2.55**	**11.9**	**O2+12.8**
Number of inseminated rats	25	25	25	25	25	25
Dams with viable fetuses	21	22	23	23	23	23
Number of implantations per dam	12.3	12.8	11.3	11.4	11.3	11.3
Food intake, pregnancy	19.9	20.0	19.1**	18.7**	17.7**	17.4**
Weight gain, pregnancy [g]	83.6	88.8	76.8	74.7**	58.7**	62.3**
Corrected weight gain [g]	20.0	23.0	19.8	19.3*	13.6**	12.5**
Corpora lutea per group	301	312	313	316	319	310
Preimplantation loss per group	42	31	53*	54*	59**	51*
Number of live fetuses per dam	11.6	12.0	10.7	10.9	10.4*	10.4*
Mean weight of fetuses [g]	3.41	3.50	3.48	3.13**	2.48**	2.83**
Mean placenta weight [g]	0.61	0.60	0.62	0.56*	0.46**	0.51**

a = air control, v = vehicle control; * = p <0.05, ** = p <0.01 in relation to air and vehicle control.

At 2.55 mg/m³ air and above, fetuses exhibited signs of retarded ossification of the phalanges, metacarpals and metatarsals, sternebrae, vertebrae, pelvis or the skull. With oxygen supplement the embryotoxic findings in the high dose group were less pronounced (Table 71).

Table 71: Summary of skeletal findings of fetuses (study 78)

Dose [mg/m³ air]	0 a.	0 v.	0.46	2.55	11.9	O2+12.8
Number of fetuses examined	126	138	128	133	124	126
Distal Phalanx – unossified (1st right %)	4.8	3.6	1.6	7.5	45.2***	10.3
Metacarpals – incompl. ossified (2nd right %)	0.8	0.0	0.8	3.0	41.1***	15.1***
Sternum – unossified (2nd segment %)	0.0	0.0	0.0	0.8	19.4***	7.9***

a = air control, v = vehicle control; *** = p <0.001

An increased incidence of malformations was also observed at levels of 2.55 mg/m³ air and above (Table 72). With the exception of the occurrence of microphthalmia and anophthalmia in the high dose groups, the nature of malformations were comparable to those in the controls of this or previous studies (hydrocephalus internus: 1/0/0/0/0/0; skeletal dysplasia of legs: 0/1/1/4/1/3; filiform tail: 0/0/0/1/0/0; spinal malformation: 0/0/0/0/2/0; rib malformation: 0/0/0/0/1/0; malformation of exoccipital bone and cervical vertebral arches: 1/0/0/0/3/0; dysplasia of exoccipital bone: 0/0/0/0/1/0; umbilical hernia: 0/0/0/0/1/0) did not indicate a specific teratogenic potential of cyfluthrin inhalation. The incidence of microphthalmia was outside the historical control values (1983-1992) (no. of foetuses per year: 2/6/3/2/5/6/3/1/1/2).

Table 72: Summary of malformations in fetuses (Holzum, 1993)

Dose [mg/m³ air]	0 a.	0 v.	0.46	2.55	11.9	O_2+12.8
Microphthalmia (Fetuses / Litters affected)	1/1	2/2	1/1	3/2	13/8**	7/5
Anophthalmia (Fetuses / Litters affected)	-	-		-	1/1	1/1
Fetuses per group (n)	243	263	245	251	239	240
Total malformed fetuses (n)	3	3	2	8	21***	10
Litters with malformations (n)	2	3	2	4	10*	7

a = air control, v = vehicle control; * = p <0.05, ** = p <0.01, *** = p <0.001

In another teratogenicity study (with two separate experiments) with inhalation exposure of cyfluthrin (study 77) a maternal and developmental NOAEL of 0.59 mg cyfluthrin/m³ air was based on reduced body weight development in the dams, reduced foetal weight (Table 73) and retarded ossification at the next higher dose of ≥1.1 mg/m³ air. In addition, at ≥4.16 mg/m³ air (+O_2) clinical signs occurred in the dams and an increased incidence of microphthalmia was noted in the offspring (see

Table 75 and Table 76).

In the dams no deaths occurred as a result of the treatment. At 4.16 mg/m³ air (+O_2) (experiment 2) and from 4.7 mg/m³ air (experiment 1) onwards clinical signs in the form of reduced motility, piloerection, ruffled/unkempt fur, irritation of the visible eye mucous membranes and labored breathing were observed (incidences in 48% of the animals at 4.16 mg/m³ air, 87% of the animals at 4.7 mg/m³ air, and 100% of the animals at 23.7 mg/m³ air). The rats with oxygen substitution tolerated the exposure better (lower intensity of the clinical signs) than the corresponding rats without the oxygen exposure.

Body weight development of dams was reduced from the dose of 1.1 mg/m³ air both during the administration and the remaining gestation period. At 4.16 mg/m³ air with oxygen substitution the body weight development was retarded only during the administration period. Both, clinical signs and the decreased body weight gain were interpreted as an indication of maternal toxicity (Table 73 and Table 74). At 1.1 mg/m³ air onwards mean foetus and placenta weights were lower, the number of runts higher (Table 73 - Table 76).

Table 73: General examinations (parental data, experiment 1) (study 77)

Dose [mg/m³ air]	**0**	**1.1**	**4.7**	**23.7**
Number of inseminated rats	30	30	30	30
Number of pregnant rats	25	29	27	29
Number of implantations	11.5	12.2	11.7	11.6
Weight gain during pregnancy [g]	75.5	66.6*	57.1**	45.6**
Number of losses of fetuses (per dam)	0.7	0.9	1.6	2.3*
Number of live fetuses	10.8	11.3	10.1	9.3
Mean weight of fetuses [g]	3.4	3.16*	2.89**	2.43**
Mean weight of placenta [g]	0.57	0.52*	0.48**	0.40**

* = p <0.05, ** = p <0.01.

Table 74: General examinations (parental data, experiment 2) (study 77)

Dose [mg/m³ air]	**0**	**0.09**	**0.25**	**0.59**	**O_2+4.16**
Number of inseminated rats	30	30	30	30	30
Number of pregnant rats	23	29	25	29	22
Number of implantations	10.7	11.4	11.2	11.0	11.2
Weight gain during pregnancy [g]	58.4	63.0	60.2	85.9	56.4
Number of losses of fetuses (per dam)	1.7	1.8	2.4	1.8	1.7
Number of live fetuses	9.0	9.6	8.8	9.2	9.5
Mean weight of fetuses [g]	3.48	3.51	3.53	3.47	3.29*
Mean weight of placenta [g]	0.61	0.61	0.62	0.58	0.56*

* = p <0.05, ** = p <0.01.

The slightly increased frequency of microphthalmia (unilateral) at 23.7 mg/m³ air (

Table 75) was outside the historical control values (6 incidences in 8 studies in 1984, 2 incidences in 15 studies in 1985) for this finding. These effects were interpreted as signs of a non-specific retardation of embryonic development and are attributed to a maternal hypoxia induced by the treatment rather to an embryotoxic potential of cyfluthrin. Accordingly, the effects were considerably less pronounced at 4.16 mg/m³ air with oxygen substitution than at 4.7 mg/m³ air without oxygen substitution. No further evidence of a teratogenic potential was found at doses up to and including the highest, clearly maternal-toxic dose.

Table 75: Anomalies (mean values / standard deviation, experiment 1) (study 77)

Dose [mg/m³air]	0	1.1	4.7	23.7
Skeletal variations	1.80 / 1.71	2.62 / 1.59	3.89* / 2.47	5.32** / 2.65
Runts	0.20 / 0.50	2.00* / 3.13	4.89** / 4.64	7.57** / 4.15
Malformations (all)	0.04 / 0.20	0.07 / 0.26	0.15 / 0.46	0.29 / 0.71
Microphthalmia: absolute number of pups	1/271	2/319	2/292	8/261

* = p <0.05, ** = p <0.01.

Table 76: Anomalies (mean values / standard deviation, experiment 2) (study 77)

Dose [mg/m³ air]	0	0.09	0.25	0.59	O_2+4.16
Skeletal variations	2.52 / 2.19	2.45 / 1.92	1.64 / 1.41	1.86 / 1.77	2.82 / 1.30
Runts	0.35 / 0.78	0.38 / 0.73	0.32 / 0.69	0.21 / 0.49	1.14* / 1.58
Malformations (all)	0.04 / 0.21	0.10 / 0.31	0.20 / 0.65	0.03 / 1.19	0.05 / 0.21
Microphthalmia: absolute number of pups	1/206	1/278	2/221	1/268	1/209

* = p <0.05, ** = p <0.01.

The data of an addendum provide explanations for the reproductive effects observed. Accordingly, the reflex bradypnoea of the dams which is compensated by hypothermia and a reduction in metabolic activity seems responsible for the impairment of intra-uterine processes.

Conclusions of the Pesticides Peer Review:

The increased frequency of microphthalmia and the proposed mode of action (secondary effect due to hypoxic conditions) were discussed during the Pesticides Peer Review Meeting 172. The existence of the proposed mode of action could not be confirmed in open literature. It was noted that with additional oxygen exposure in the high dose group, the incidence of microphthalmia was lower than without oxygen supplementation, but remained higher than control values (study 78, for data refer to table 54). Therefore, the mode of action proposed by the Dossier submitter was not supported by the meeting and the finding of microphthalmia in inhalation developmental toxicity studies was regarded potentially relevant to humans. A proposal for classification as developmental toxicant category 2 (H361d "Suspected of damaging the unborn child") was agreed by majority of experts at this meeting.

Effects via lactation:

After Annex I inclusion according to Directive 91/414/EC (concerning the placing of plant protection products on the market) a developmental neurotoxicity screening study with beta-cyfluthrin in rats has been conducted. The study was submitted for renewal procedure for beta-cyfluthrin under Regulation (EC) No 1107/2009 and was previously not evaluated on EU level (study 80, see Table 82).

Table 77: Summary of developmental neurotoxicity study

Table Study	Dose levels	Test substance	NO(A)EL	Targets / Main effects	Reference
Developmental Neurotoxicity Screening Study in Rats; diet (GLP: yes,OECD TG 426)	0-30-125-200 ppm (equal to 0-2.4-11.0-17.8 mg/kg bw/d during gestation and 0-5.9-25.4-40.9 mg/kg bw/d during lactation) (Wistar rats) (30 females/group)	beta-cyfluthrin batch-No. 8030130/380566042, purity: 95.1-97.6%; vehicle: none (covered in diet)	125 ppm (equivalent to 11 mg/kg bw/d during gestation)	Maternal: 200 ppm: Lower bw development during gestation and lactation Offspring: 200 ppm: Reduced pup weight gain, FOB*: minimal resistance during handling, reduced startle response	study 80 †

* FOB: Functional observation battery
†Key study

Technical grade beta-cyfluthrin was administered via the diet from gestation day (GD) 0 through lactation day (LD) 21 to mated female Wistar rats at nominal concentrations of 0, 30, 125 and 200 ppm (equal to 0, 2.4, 11.0 and 17.8 mg/kg/day, respectively during gestation and 0, 5.9, 25.4 and 40.9 mg/kg/day, respectively during lactation). The adult males served only as "breeders" and were not exposed to the test substance or included in any tests.

On postnatal day (PND) 4, litters with a minimum of eight pups, including at least three per sex, were culled to yield, as closely as possible, four males and four females. Subsets of surviving offspring, representing at least 20 litters per level, were subjected to evaluation using the following observations and measurements - detailed clinical observations (an abbreviated functional observational battery), preputial separation or vaginal patency, body weight, food consumption, body temperature, automated measures of activity (figure-eight maze), acoustic startle habituation, learning and memory (passive avoidance after weaning and a water maze task on PND 60) and an ophthalmic examination.

Neural tissues were collected from 10/sex/dietary level (representing approximately 20 litters) on PND 21 (brain only) and at study termination (approximately 75 days of age) for microscopic examination and morphometry. The concentration of beta-cyfluthrin in the whole-brain from the dams (LD 21) and offspring (PND 4 and PND 21) was also measured to verify exposure.

In the maternal animals there were no deaths prior to terminal sacrifice. Lower body weight development during gestation day 6 was noted in high dose dams (200 ppm). During lactation (days 0-21) body weight development and food consumption was reduced in dams of the 200 ppm group.

During lactation hair loss was noted in few dams of groups 3 and 4 (125 and 200 ppm). The FOB was unaffected in dams during gestation and lactation until PND 21. Pup weight gain was reduced from days 11 to day 21 in pups of the 200 ppm group. Further litter data were not affected by the treatment (

Table 78).

Table 78: Body weight development of pups during lactation [g ± SE]

PND	Dietary level [ppm]							
	0		30		125		200	
	Males	Females	Males	Females	Males	Females	Males	Females
0	5.8±0.08	5.5±0.09	5.7±0.09	5.4±0.08	5.8±0.08	5.5±0.07	5.7±0.09	5.4±0.10
4	9.7±0.22	9.3±0.24	9.2±0.19	8.9±0.17	9.6±0.17	9.2±0.18	9.0±0.21	8.6±0.21
11	24.7±0.48	23.5±0.48	23.3±0.57	23.0±0.55	23.9±0.36	23.3±0.36	22.2±0.21**	21.4±0.54*
17	39.0±0.64	36.9±0.64	37.0±0.67	36.2±0.65	37.3±0.52	36.3±0.52	35.2±0.72**	34.0±0.75**
21	49.6±0.85	46.7±0.87	46.5±0.79*	45.3±0.75	47.1±0.65	45.6±0.65	44.3±0.83**	42.9±0.86**

Dunnett's test*p≤0.05, **p≤0.01

In the FOB for pups on PND 4, minimal resistance during handling was noted for pups of the high dose group (200 ppm). No further changes were noted in animals up to PND 60. Automated measures for motor and locomotor activity were not affected by treatment at any dietary level. There were no compound-related ophthalmic findings. Reduced response amplitude following acoustic startle habituation was observed in male high-dose pups at PND 22. This finding was associated with reduced body weight. It was not observed at later time points, in females or other dose groups. There were no effects of treatment on developmental landmarks (balano-preputial separation or vaginal patency) (Table 79 and Table 80).

Table 79: Developmental landmarks

	Dietary level [ppm]			
	0	30	125	200
Preputial separation				
Age at landmark [days ± SE]	43.6±0.34	43.9±0.29	43.8±0.32	44.2±0.35
BW at landmark [g ± SE]	185±2.0	178±1.7*	178±1.7*	171±1.8**
Vaginal opening				
Age at landmark [days ± SE]	34.0±0.27	35.0±0.25*	34.4±0.23	34.6±0.24
BW at landmark [g ± SE]	106±1.7	107±1.3	105±1.4	101±1.1*
Pupil constriction				
Pups reaching criteria [%]	100	100	100	100

Dunnett's test, Fisher's exact test *p≤0.05, **p≤0.01

Beta-cyfluthrin was detected in brain tissue from pups on both days measured (PND 4 and PND 21) at all dietary levels, with the concentration increasing in proportion to the dietary concentration (Table 80). These findings provide clear evidence of exposure during lactation.

Table 80: Concentration of beta-cyfluthrin in whole-brain tissue

Dietary level [ppm]	Tissue level of beta-cyfluthrin [ppm]		
	Pups (PND 4)[1]	Pups (PND 21)	Dams (LD 21)
0	0.000	0.002	0.000
30	0.004	0.006	0.006
125	0.016	0.024	0.026
200	0.026	0.034	0.046

Based on 16-22 pups (representing a minimum 16 litters) and 18-22 dams per group.

[1] Samples were pooled to provide adequate amounts for analysis.

Compound-related gross lesions were not evident in males or females at terminal sacrifice. There were no effects on brain weight, brain morphometry or histology of brain, neural tissues or skeletal muscle at study termination.
Treatment did not affect reproduction parameters, including the fertility index (Table 81).

Table 81: Reproductive parameters

	Dietary level [ppm]			
	0	**30**	**125**	**200**
No. of animals cohoused	30	30	30	30
No. of animals mated	30	30	30	30
Mating index	100.0	100.0	100.0	100.0
Fertility index	86.7	96.7	96.7	86.7

The overall NOAEL was 125 ppm (equivalent to 11.0 mg/kg bw/day during gestation) based on effects on body weight and food consumption in high-dose dams and effects on body weight and startle response in high-dose pups at 17.8 mg/kg bw/day.

Table 82: Detailed findings in study 80

Detailed study findings:						
Endpoint	**Generation**	**Dietary Level (ppm)**				**Comment**
		0	30	125	200	
Clinical observations during gestation						
No. animals/group	F0	30	30	30	30	Compound-related clinical signs were not evident at any dietary level. No mortality occurred.
No remarkable clinical observations		30	30	29	27	
Lacrimal stain, red		0	0	0	1	
Hair loss		0	0	1	2	
Clinical observations during lactation						
No. animals/group	F0	26	29	29	26	Compound-related clinical signs were not evident at any dietary level. No mortality occurred.
No remarkable clinical observations		26	29	28	24	
Hair loss		0	0	1	2	
Clinical observation during PND 0-21						
No. litters examined	F1	26	29	29	26	No compound-related signs were observed during lactation in males or females at any dietary level. Incidental findings that were evident on occasion in
Bruise on face/back/body		5/2/1	7/3/0	5/1/2	5/6/0	

Detailed study findings:						
Endpoint	**Generation**	**Dietary Level (ppm)**				**Comment**
		0	30	125	200	
						individuals from various dose groups, including controls, included bruising, raised area on the dorsal neck (one high-dose pup), wounds/bite marks or cuts, a missing hindfoot (one control) and a swollen forelimb (one high-dose pup).
Clinical observations post weaning	F1					Compound-related clinical signs were not evident at any dietary level.
Alopecia (back)	Male/ female	-/1	-	-	1/2	
Lesion, sore	Male/ female	-	1/-	2/-	4/-	
Lesion, scab	Male/ female	4/0	2/0	2/1	4/5	
Dehydrated, bod	Male/ female	-	-	3/-	2/-	
Dead	Male/ female	-	-	1/-	2/-	
Nasal stain	Male/ female	-	-/1	-	-	
Urine/perianal stain	Male/ female	-/3	-/1	-	-	
Exophthalmos	Male/ female	-	-/1	-	-	
Eye, small, left	Male/ female	-	-	1/-	-	
Functional Observational Battery	F0					Compound-related functional observations were not evident at any dietary level.
Rearing Mean ± S.D.	Females	3.1±1.9	2.7±1.7	3.7±1.8	2.4±1.7	
Defecation Number of Boluses Mean ± S.D.		0.7±1.3	0.3±0.7	0.6±1.0	0.6±1.0	
Urination Number of Pools Mean ± S.D.		1.2±1.2	1.3±1.6	1.1±1.3	0.8±0.9	

4.10.2.2 Human information

Toxicity via lactation:

Human data are available for monitoring of pesticide residues in breast milk. Measurements in humans show that pesticide residues, including cyfluthrin, were detected in breast milk samples (Anupama et al., 2014; Bouwman and Kylin, 2009; Bouwman et al., 2006; Feo et al., 2012; Sereda et al., 2009). Anupama et al. (2014) reported that cyfluthrin was the leading pesticide detected in breast milk contributing 31.28% to the total residue load. Infants under malaria control conditions in South Africa are exposed to combinations of chemicals, i.e. cyfluthrin, alpha-cypermethrin, DDT, deltamethrin that would have deleterious effects if the intakes were high enough. Levels of up to 459 µg/L whole milk were recorded for cyfluthrin, of up to 28 µg/L whole milk for alpha-cypermethrin, of up to 725 µg/L whole milk for DDT and of up to 83 µg/L whole milk for deltamethrin (Bouwman and Kylin, 2009).

Organochlorine (i.e. DDT and its metabolites, fipronil, endosulfan), organophosphate (i.e dimethoate, carbaryl, chlorpyrifos) and synthetic pyrethroids (i.e. cyfluthrin, alpha-cyhalothrin and deltamethrin) pesticides are widely used for the purpose of enhancing food production and improving health by destroying insects and pests of food crops and vectors of human and animal diseases like malaria, dengue, encephalitis, filariasis etc. However, accumulation of these pesticides in the food chain results in accumulation in human (and animal) body. Residues of these pesticides get accumulated in the lipid-rich tissue in the body and and are finally excreted in the mother's breast milk. The results of Anupama et al. (2014), Bouwman and Kylin (2009), Bouwman et al. (2006) indicated that the infant daily intake of these pesticides from some of the breast-milk samples exceeded health-based acceptable levels, like the respective ADI value. Thus, a risk of infants to these pesticides cannot be excluded. Adverse health effects of breast-milk fed infants are not reported in these publications.

Likewise, in the Renewal Assessment Report (RAR, 2015) of beta-cyfluthrin as an active substance in plant protection products, and in the Summary Report of cyfluthrin for use in veterinary medicines (2002) certain investigations have shown that residues after oral administration of cyfluthrin and beta-cyfluthrin were found in lactating cows and goats, respectively (study 81, 82). See also Chapter 4.10.4 Summary and discussion of reproductive toxicity.

4.10.3 Other relevant information

No data available.

4.10.4 Summary and discussion of reproductive toxicity

There are no appropriate epidemiological studies available on developmental effects in humans. Hence, classification with Category 1A according CLP regulation is not possible.

Fertility:

Under the conditions of the two-generation reproductive toxicity study, cyfluthrin had no effect on fertility when administered via the diet to rats up to 400 ppm, the highest dose tested.

Development:

The prenatal developmental toxicity of beta-cyfluthrin and cyfluthrin was investigated in rats and rabbits and the studies were considered acceptable.

In the inhalational teratogenicity studies in rats with cyfluthrin (study 77, 78), the increased frequency of malformations (microphthalmia, anophthalmia, bone malformations) observed in the offspring at 11.9, 12.8 (with oxygen supplement), and 23.7 mg cyfluthrin /m^3 air was considered a secondary effect following hypoxic conditions in the dams Due to the irritating properties of the test substance at these dose levels a reflex bradypnoea occurred in the dams which was compensated by hypothermia and a reduction in metabolic activity. In addition, an increased incidence of resorptions occurred at a dose level of 23.7 mg/m^3 (study 77). It can be assumed that the occurrence of the mentioned malformations, especially microphthalmia, in the offspring does not represent a direct toxic effect of the test substance. This assumption is supported by reproductive toxicity studies with orally administered beta-cyfluthrin/cyfluthrin, which are systemically available by oral absorption (60 % (beta-cyfluthrin) and 90 % (cyfluthrin)). After oral administration no treatment-related malformations were observed.

Even though some of the observed findings in the dams were severe findings (such as clinical signs, motor disturbances and/or gait abnormalities), they were considered to represent acute toxic/neurotoxic effects of beta-cyfluthrin/cyfluthrin. Due to intensive metabolism and rapid excretion of beta-cyfluthrin/cyfluthrin (see Chapter 4.1 Absorption, distribution, metabolism and excretion in mammals), daily administrations of beta-cyfluthrin/cyfluthrin are considered to represent a sequence of acute intoxications.

Due to signs of respiratory irritation observed in humans and in appropriate animal teratogenicity studies after cyfluthrin exposure via inhalation is proposed to classify and label beta-cyfluthrin/cyfluthrin accordingly for respiratory irritating effects (STOT SE; 3 H335 May cause respiratory irritation).

Manifestations of developmental toxicity seen in rats and rabbits were accompanied by maternal toxicity. Abortion was observed in two (top dose) rabbits, and one dam resorbed its implants completely (study 74). From 60 mg/kg bw/d an increase in the number of post-implantative resorptions was the only observed change in rabbits interpretable as a sign of reproduction toxicity (study 75). Taken together, based on the small number of animals affected, these findings are considered not severe enough to justify a classification in Category 2 (H361d).

In a developmental neurotoxicity screening study with beta-cyfluthrin in rats (study 80), no effect on developmental landmarks (balano-preputial separation or vaginal patency) and on reproduction parameters, including the fertility index in the offspring were noted.

Lactation:

The NOAEL for parental toxicity was established at 50 ppm, based on reduced body weights of F_1 males at and above 125 ppm. At 400 ppm, clinical signs of neurotoxicity (splayed hind limbs) were observed in F_0 and F_1 females during lactation and body weights and food consumption were reduced in both sexes. The NOAEL for offspring toxicity was established at 50 ppm, based on increased incidences of coarse tremors and decreased pup body weights at and above 125 ppm during the lactation period.

Beta-cyfluthrin has lipophilic properties and dependent on the extent of exposure, the substance gets accumulated in the lipid-rich tissue of the breast and transfer into human or animal breast milk will

occur.

No measurements of beta-cyfluthrin concentration in the rat milk after exposure have been provided and according to our literature research, no such information does exist.

Measurements of beta-cyfluthrin concentration in whole-brain tissue were performed in the developmental neurotoxicity study in rats (study 80). Beta-cyfluthrin was detected in brain tissue from pups on both days measured (PND 4 and PND 21) at all dietary levels, with the concentration increasing in proportion to the dietary concentration. These findings provide clear evidence of exposure of the pups during lactation and that beta-cyfluthrin can reach the pups via the dam’s milk.

Additionally, residues of cyfluthrin were detected in human breast milk samples. It can be concluded that the presence of adverse effects in the offspring in the 2-generation toxicity study in rats during lactation was due to transfer of cyfluthrin and/or its metabolite(s) in the milk, which will result in a proposal for classification and labelling (see chapter 4.10.6).

4.10.5 Comparison with criteria

Toxicological result	Hazard category for lactation effects
Beta-cyfluthrin has lipophilic properties and residues of cyfluthrin were detected in human breast milk samples; Increased incidences of coarse tremors and decreased pup body weights at and above 125 ppm cyfluthrin during the lactation period was observed in the rat 2-generation toxicity study.	EFFECTS ON OR VIA LACTATION Effects on or via lactation are allocated to a separate single category. It is recognised that for many substances there is no information on the potential to cause adverse effects on the offspring via lactation. However, substances which are absorbed by women and have been shown to interfere with lactation, or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, shall be classified and labelled to indicate this property hazardous to breastfed babies. This classification can be assigned on the: (a) human evidence indicating a hazard to babies during the lactation period; and/or (b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or (c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.

4.10.6 Conclusions on classification and labelling

Cyfluthrin exposure through the milk is considered to be the main determinant of offspring neurotoxicity in the 2-generation toxicity study in rats and it is proposed to classify beta-cyfluthrin as a reproductive toxicant in category for effects on or via lactation.
Classification and labelling for reproductive toxicity according to Regulation (EC) No 1272/2008 (GHS): Lact H362: May cause harm to breast-fed children.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The DS presented a two-generation study in rats with cyfluthrin, several pre-natal developmental toxicity (PNDT) studies with cyfluthrin (oral and inhalation studies in the rat, oral studies in the rabbit), a rat oral PNDT study with beta-cyfluthrin and a developmental neurotoxicity (DNT) study in rats with beta-cyfluthrin.

Fertility

The DS proposed no classification based on lack of effects on fertility in the two-generation study with cyfluthrin (study 70).

Development

The DS discussed increased incidence of microphthalmia and other developmental effects in the rat inhalation PNDT studies with cyfluthrin (study 77 and 78). They considered the findings as secondary to the hypoxic condition of the dams (hypoxia due to decreased respiratory rate resulting from sensory irritation) since oxygen supplementation reduced the incidences and no treatment-related malformations were observed in oral studies.

Increased post-implantation loss was observed in one of the rabbit studies with cyfluthrin (study 75) but the DS did not consider this finding sufficient for classification. Retarded ossification and reduced foetal weight were observed in the rat PNDT study with beta-cyfluthrin (study 76) in the presence of maternal toxicity. No other effects related to developmental toxicity were found in the available studies.

Overall, the DS proposed no classification for effects on development.

Lactation

The DS proposed classification with Lact.; H362 based on increased incidence of coarse tremors in pups (from postnatal day (PND) 5 to 18) and decreased pup weights during lactation in the two-generation study with cyfluthrin (study 70). The tremors in pups were observed not only at the top dose of 400 ppm associated with neurotoxicity in dams (splaying of the hind limbs) but also at the mid-dose of 125 ppm without maternal toxicity. Transfer of the substance from the dams to the pups via milk was confirmed by detection of beta-cyfluthrin in pup brains on PND 4 in the DNT study (study 80). The DS also pointed out that cyfluthrin residues were detected in human breast milk samples.

Comments received during public consultation

Comments on the reproductive toxicity classification of cyfluthrin and/or beta-cyfluthrin were received from five MSCAs and two industry commenters (one manufacturer and one downstream user).

The DS proposal for no classification for fertility was supported by two MSCAs.

As to the development, two MSCAs proposed classification in Category 2 mainly based on microphthalmia in the rat inhalation PNDT studies. They did not consider the proposed MoA sufficiently demonstrated as oxygen supplementation did not reduce the incidence of microphthalmia down to the control levels. One of the MSCAs suggested that the absence of microphthalmia in the oral PNDT studies could be a consequence of first pass effect.

One MSCA and the industry commenters supported the DS's proposal of no classification for development. The manufacturer summarised the available toxicokinetic data indicating that plasma concentrations of cyfluthrin or beta-cyfluthrin (parent substances) in the negative rat PNDT studies via gavage were much higher than those measured in the inhalation studies where eye malformations were observed. Industry also referred to a recent publication reviewing the regulatory and mechanistic inhalation studies with cyfluthrin and beta-cyfluthrin (Pauluhn, 2018).

Classification with Lact.; H362 was supported by four MSCAs. The manufacturer put forward arguments against classification. They argued that the tremors in the neonates are transient and characteristic of acute neurotoxicity associated with Type II pyrethroids, when threshold concentrations of the parent compound reach the brain. Neonatal rats are

more sensitive than adults to acute toxicity of Type II pyrethroids most likely due to limited metabolic capacity (as indicated e.g. by a study with deltamethrin where the LD_{50} values differed 7-fold between weanlings and adults but the brain concentrations at the LD_{50} were approximately the same; Sheets, 1994). Industry mentioned an ongoing research on metabolism of pyrethroids. According to their interpretation of the available data, pyrethroids are metabolised primarily by P450 enzymes in rats and carboxylesterases in humans, with carboxylesterases developing rapidly after birth in humans. Based on this information the manufacturer proposed that human infants are not more sensitive than the mothers to the neurotoxicity of Type II pyrethroids. Further, industry presented a risk-based argument to support their case against classification: humans, including lactating females, would never be exposed to the high concentrations of cyfluthrin or beta-cyfluthrin required to overwhelm the metabolising capacity of the sensitive neonate rat. The DS replied that the argumentation via metabolic capacity of carboxylesterases is based on a lot of speculation, which cannot be used to exclude a hazard for human health.

Assessment and comparison with the classification criteria

Adverse effects on fertility and sexual function

A two-generation study in rats conducted according to OECD TG 416 (1983) is available for cyfluthrin (study 70; GLP; started in 1993; top dose 400 ppm). RAC notes that the study did not investigate some of the parameters added into the test guideline in 2001 (e.g. sperm parameters, puberty onset).

In addition, a follow-up two-generation study (study 71; top dose 50 ppm) was conducted to clarify whether 50 ppm in the previous study was a no-observed-adverse-effect level (NOAEL) for effects in the offspring. No treatment-related effects were observed in this study. It was concluded that the transient pup body weight reductions seen in the first study were not treatment-related, and hence the 50 ppm NOAEL was confirmed.

Information related to fertility and sexual function can also be obtained from a DNT study in rats with beta-cyfluthrin (study 80; OECD TG 426, GLP; top dose 200 ppm).

Two-generation study in rats with cyfluthrin (study 70)

Cyfluthrin was administered to Sprague-Dawley rats at dietary concentrations of 0, 50, 125 and 400 ppm, corresponding to 0, 3/4, 9/10 and 29/33 mg/kg bw/d (m/f), respectively, except for females during lactation when the test substance intake approximately doubled (to 0, 7, 19 and 59 mg/kg bw/d). Top dose females of both generations displayed clinical signs of neurotoxicity (splaying of the hind limbs; incidence 15/29 and 9/25 in F0 and F1 respectively) during lactation only, probably due to increased test substance intake during this period. Sucklings were found to be more sensitive than dams, with coarse tremors starting already from 125 ppm; the tremors in pups are discussed under lactation.

There were no effects on reproductive parameters (oestrus cycle staging; pre-coital interval; mating, fertility and gestation indices; gestation length; number of implantation sites; birth index). No treatment-related gross or histopathological lesions were observed in the reproductive organs.

Developmental neurotoxicity study in rats with beta-cyfluthrin (study 80)

Beta-cyfluthrin was administered to female Wistar rats via diet from gestation day (GD) 0 to lactation day (LD) 21. The top dose of 200 ppm (18 mg/kg bw/d during gestation and

41 mg/kg bw/d during lactation) caused body weight reduction in pups (none at birth, by ca. 10% on PND 11, no further decrease compared to controls). Maternal body weight gain and food consumption during gestation were not affected.

There was no effect on reproduction parameters and no effect on puberty onset in this study.

No effects on reproductive parameters or reproductive organs were observed in the available studies with cyfluthrin and beta-cyfluthrin. **No classification is warranted for adverse effects on fertility and sexual function**.

Adverse effects on development

Several types of studies are available to provide information on developmental toxicity of cyfluthrin and beta-cyfluthrin: rat PNDT studies via gavage, rabbit PNDT studies via gavage, rat PNDT studies via inhalation, a rat dietary DNT study and a rat dietary two-generation study. Developmental findings potentially relevant for classification were observed in one of the rabbit PNDT studies (increased post-implantation loss in study 75) and in the rat PNDT studies via inhalation (increased incidence of microphthalmia in studies 77 and 78).

Rat PNDT studies via gavage with cyfluthrin and beta-cyfluthrin (studies 72, 73 and 76)

In study 72 cyfluthrin was administered to BAY:FB rats in PEG 400 from GD 6 to 15. The top dose of 30 mg/kg bw/d induced clinical signs of neurotoxicity (high-stepping gait, ataxia) in several dams. No developmental toxicity was observed.

In study 76 beta-cyfluthrin was administered to Wistar rats in aqueous Cremophor from GD 6 to 15. Maternal toxicity at the top dose of 40 mg/kg bw/d included mortality (3 out of 26 animals), clinical signs (hypoactivity, locomotor incoordination, salivation; all or almost all animals, depending on the effect) and reduced body weight gain (net body weight gain reduced by 14 g). Developmental toxicity at the top dose was limited to reduced foetal weight (by 9%) and delayed ossification. The top dose is considered to exceed the maximum tolerated dose (MTD). The mid-dose of 10 mg/kg bw/d caused slight maternal toxicity (reduced body weight gain) and no developmental toxicity.

In study 73 cyfluthrin was administered to Wistar rats in aqueous Cremophor from GD 6 to 15. No developmental or maternal toxicity was observed up to the top dose of 10 mg/kg bw/d. Lack of maternal toxicity at the top dose is considered a significant limitation of this study.

In summary, no effects warranting classification were observed in the available rat PNDT studies via gavage.

Rabbit PNDT studies via gavage with cyfluthrin (studies 74 and 75)

In study 74, cyfluthrin was administered to Himalayan rabbits in aqueous Cremophor from GD 6 to 18. Two dams aborted on GD 25 and 28 and one dam completely resorbed her three implants at the top dose of 45 mg/kg bw/d. Reporting of the study in the brief study report available to RAC is rather limited and individual data for most parameters are not provided. It is thus not clear whether two abortions and one complete resorption in this study represent maternal or developmental toxicity. Post-implantation loss was 6%, 14%, 17% and 20% at 0, 5, 15 and 45 mg/kg bw/d respectively (the two abortions at the top

dose excluded, the dam with total litter loss included). No treatment-related malformations were observed.

In study 75 cyfluthrin was administered to Chinchilla rabbits in corn oil from GD 6 to 18 at 0, 20, 60 and 180 mg/kg bw/d. Maternal food consumption during the treatment period was significantly reduced by 41% and 27% at the high and mid-dose respectively. Corrected weight gain was not affected as the dams were able to compensate for the initially impaired weight gain by the end of the study (dosing until GD 18, sacrifice on GD 28). Post-implantation loss was increased approx. 3-fold at the top dose, above the historical control range (only foetus-based historical control data (HCD) available: mean 8%, SD 5%, range 2-20%; current study 11%, 11%, 20%, 29% at 0, 20, 60, 180 mg/kg bw/d respectively; HCD comprise 13 studies within 3 years before the current study). There was no strong correlation between food consumption during the treatment period and post-implantation loss at the level of individual data (see 'Supplemental information in the Background document'). Still, this does not exclude some contribution of maternal toxicity to the observed effect on embryonic/foetal viability. No treatment-related increase in malformations or variations was observed in this study. Foetal weights were not decreased.

Rabbit PNDT study 75				
Dose (mg/kg bw/d)	**0**	**20**	**60**	**180**
Total no. of females	16	16	16	16
Pregnant	16	13	16	16
Total litter loss	0	0	0	1
Food consumption GD 6-19 (g/animal/day)	131	121	96*	77*
Post-implantation loss[a] (%; ±SD)	10 (±11)	14 (±21)	19 (±15)	31/26[b] (±29/23)
Embryonic resorptions[a] (%; ±SD)	4 (±7)	10 (±21)	12 (±15)	22/17[b] (±30/22)
Implantation sites (mean/dam)	12.1	9.8	11.4	12.4/12.2[b]
Live foetuses (mean/dam)	10.8	8.8	9.2	8.9/8.3[b]

[a] The study report provides only foetus-based values; litter-based values (*i.e.* mean of % losses in the individual litters) have been calculated by RAC. As to statistical evaluation, post-implantation loss is not significant (at p=0.05) in Kruskal-Wallis and significant in parametric ANOVA, embryonic resorptions not significant in Kruskal-Wallis nor parametric ANOVA (top dose dam with total litter loss included)
[b] Including/excluding the dam with total litter loss
* Stat. significant, p≤0.05; stat. analysis of food consumption conducted by RAC (ANOVA followed by Dunnett's test)

<u>Two-generation study with cyfluthrin and DNT study with beta-cyfluthrin (studies 70 and 80)</u>

No developmental toxicity was observed in these two dietary studies. Marked pup body weight reductions observed in the two-generation study (study 70) from PND 4 are discussed under lactation.

<u>Rat PNDT studies via inhalation with cyfluthrin (studies 77 and 78)</u>

Study 77 comprised two experiments that served as pilot studies to the main study, study 78.

In the first experiment of study 77, Wistar rats (Bor:WISW) were exposed to cyfluthrin in ethanol/PEG 400 head-nose only from GD 6 to 15 for 6 hours per day at concentrations of 0 (vehicle), 1.1, 4.7 and 23.7 mg/m3. Clinical signs (dyspnea, reduced motility, piloerection, ruffled/unkempt fur, irritation of the visible eye mucous membranes) were observed from 4.7 mg/m^3. Body weight gain of the dams was reduced at all concentrations; part of the reduction is due to lower foetal weights (foetal weights were reduced by up to 29%). Post-implantation loss was increased at the top concentration in the presence of maternal toxicity. Incidence of microphthalmia was increased at the top concentration; all cases were unilateral. According to HCD (studies within two years before the current study), microphthalmia occurred in controls of 5 out of 23 studies (incidences per group: 1, 1, 4, 1, 1).

Rat inhalation PNDT study 77, 1st experiment				
Concentration (mg/m^3)	**0 (vehicle)**	**1.1**	**4.7**	**23.7**
Pregnant rats	25	29	29	28
Incidence of dyspnea	0	0	5	20
Incidence of piloerection	0	0	25	28
Bw gain[1] during pregnancy (g)	76	67*	57**	46**
Number of implantations per dam	11.5	12.2	11.7	11.6
Number of live foetuses per dam	10.8	11.3	10.1	9.3
Post-implantation loss (absolute; mean ± SD)	0.7 (±1.0)	0.9 (±1.2)	1.6 (±3.1)	2.3* (±2.5)
Total number of foetuses	271	329	292	261
Foetal weight (g)	3.40	3.16*	2.89**	2.43**
No. of foetuses for skeletal examination (mean)	7.5	7.9	7.6	6.6
Skeletal variations (absolute; mean ± SD)	1.8 (±1.7)	2.6 (±1.6)	3.9* (±2.5)	5.3** (±2.7)
Microphthalmia, foetal (litter) incidence	1 (1)	2 (2)	2 (2)	8 (5)
All malformations, foetal (litter) incidence	1 (1)	2 (2)	4 (3)	9 (5)
All malformations (mean ± SD)	0.04 (±0.20)	0.07 (±0.26)	0.15 (±0.46)	0.29 (±0.71)

Statistically significant difference from control: *, p<0.05; **, p<0.01

[1] body weight not corrected for gravid uterine weight

The second experiment of study 77 used concentrations of 0 (vehicle), 0.09, 0.25, 0.59 and 4.16 mg/m^3. The test atmosphere at the top concentration of 4.16 mg/m^3 was enriched in oxygen (30% v/v instead of 21% v/v). The purpose of oxygen enrichment was to investigate whether the developmental effects at 4.7 mg/m^3 in the first experiment could be related to foetal hypoxia. The clinical signs at 4.16 mg/m^3 + O_2 were less pronounced than those at 4.7 mg/m^3 in the first experiment, as was foetal toxicity (foetal weight reduction 5% instead of 15%, no increase in skeletal variations vs. a two-fold increase). No increase in microphthalmia was observed at 4.16 or 4.7 mg/m^3 in either experiment.

Rat inhalation PNDT study 77, 2nd experiment					
Concentration (mg/m³)	**0 (vehicle)**	**0.09**	**0.25**	**0.59**	**4.16+O_2**
Pregnant rats	23	29	25	29	22
Incidence of dyspnea	0	0	0	0	0
Incidence of piloerection	0	0	0	0	11
Bw gain during pregnancy (g)	58	63	60	59	56
Number of implantations per dam	10.7	11.4	11.2	11.0	11.2
Number of live foetuses per dam	9.0	9.6	8.8	9.2	9.5
Post-implantation loss (absolute; mean ± SD)	1.7 (±2.0)	1.8 (±2.4)	2.4 (±2.4)	1.8 (±1.6)	1.7 (±2.2)
Total number of foetuses	206	278	221	268	209
Foetal weight (g)	3.48	3.51	3.53	3.47	3.29*
No. of foetuses for skeletal examination (mean)	6.3	6.7	6.2	6.4	6.6
Skeletal variations (absolute; mean ± SD)	2.5 (±2.2)	2.5 (±1.9)	1.6 (±1.4)	1.9 (±1.8)	2.8 (±1.3)
Microphthalmia, foetal (litter) incidence	1 (1)	1 (1)	2 (2)	1 (1)	1 (1)
All malformations, foetal (litter) incidence	1 (1)	3 (3)	5 (3)	1 (1)	1 (1)
All malformations (mean ± SD)	0.04 (±0.21)	0.10 (±0.31)	0.20 (±0.65)	0.03 (±1.19)	0.05 (±0.21)

Statistically significant difference from control: *, p<0.05; **, p<0.01

The main study (study 78), conducted seven years after the pilot studies, employed concentrations of 0 (air), 0 (vehicle), 0.46, 2.55, 11.9 mg/mg^3 and 12.8 mg/m^3 + O_2 (39% v/v). Since repeat dose and mechanistic inhalation studies performed prior to study 78 revealed strong effects on respiration and body temperature, measurements of lung function (in a plethysmograph, GD 6) and rectal temperature (GD 6 and 13) were also included in study 78. However, as these measurements could induce stress-related effects difficult to quantify, lung function and body temperature were only measured in satellite animals (5/group, exposure GD 6-13) not subject to foetal examination. These satellite animals were also used for determination of plasma levels of cyfluthrin (immediately after exposure on GD 13).

Clinical signs (e.g. ruffled fur, retarded breathing) were present mainly at the top concentrations (11.9 and 12.8 mg/m^3). Respiratory volume at the top concentrations was reduced ca. 2.5-fold compared to controls. Body temperature was reduced by ca. 4°C after the first exposure at the top concentrations irrespective of oxygen supplementation; the difference on GD 13 was smaller, approx. 3°C and 2°C without and with oxygen supplementation, respectively. Foetal weight was significantly reduced from 2.55 mg/m^3. At the top concentrations, foetal weight reduction, delayed ossification (phalanges,

metacarpals, metatarsals, sternebrae, vertebrae, pelvis, skull) and increased incidence of microphthalmia were observed both without and with oxygen supplementation, but effects in the oxygen-supplemented group were weaker (foetal weight reduction 17% vs. 27%, lower incidence of reduced ossification, lower incidence of eye malformations). The incidence of microphthalmia was not clearly related to the occurrence of clinical signs at the level of individual data (which is not surprising given that clinical signs occurred in most animals at the top concentrations). Respiratory rate and rectal temperature were only measured in satellite animals. According to the HCD (1988-1992, *i.e.* within five years before the current study, the same strain), microphthalmia occurred in 9 out of 25 studies, maximum incidence per study was altogether three foetuses, distributed in two litters.

Rat inhalation PNDT study 78						
Concentration (mg/m^3)	**0 (a.)**	**0 (v.)**	**0.46**	**2.55**	**11.9**	**12.8+O_2**
Dams with implantations	21	22	24	24	23	23
Dams with viable foetuses	21	22	23	23	23	23
Incidence of retarded breathing	0	0	0	0	17	10
Incidence of ruffled fur	0	0	0	1	19	21
Food intake, pregnancy (g/day)	20	20	19**	19**	18**	17**
Bw gain during pregnancy (g)	84	89	77*	75**	59**	62**
Corrected bw gain (g)	20	23	20	19*	14**	13**
Respiratory rate (breath/min), satellite animals	143	148	115	107	111	89
Respiratory minute volume (mL/min/kg), satellite animals	1520	1680	1200	1100	710	650
Rectal temperature after first exposure (°C), satellite animals	37.6	37.0	36.0*	34.4	32.9**	32.6**
Rectal temperature after exposure on GD 13 (°C), satellite animals	37.6	38.5**	38.0	37.2	34.7*	36.1
Number of implantations per dam	12.3	12.8	11.3	11.4	11.3	11.3
Number of live foetuses per dam	11.6	12.0	10.7	10.9	10.4*	10.4*
Post-implantation loss per dam (absolute)	0.8	0.8	0.7	0.5	0.9	0.8

Foetal weight (g)	3.41	3.50	3.48	3.13**	2.48**	2.83**
Distal phalanx (forelimb) unossified, 1st right (%)	4.8	3.6	1.6	7.5	45.2**	10.3
Metacarpal incompletely ossified, 2nd right (%)	0.8	0.0	0.8	3.0	41.1**	15.1**
Sternum unossified, 2nd segment (%)	0.0	0.0	0.0	0.8	19.4**	7.9**
Microphthalmia; foetuses (litters)	1 (1)	2 (2)	1 (1)	3 (2)	13 (8)	7 (5)
Anophthalmia; foetuses (litters)	0	0	0	0	1 (1)	1 (1)
Eye malformations; foetuses (litters)	1 (1)	2 (2)	1 (1)	3 (2)	14 (9)	7 (5)
Foetuses per group	243	263	245	251	239	240
All malformations, foetal (litter) incidence	3 (2)	3 (3)	2 (2)	8 (4)	21 (10)	10 (7)

Statistically significant difference from control: *, $p<0.05$; **, $p<0.01$

An increase in microphthalmia was observed only at concentrations apparently causing strong sensory irritation to which the maternal animals responded with pronounced physiological changes. Pauluhn (2018) proposed a MoA for the developmental effects observed in the inhalation PNDT studies with cyfluthrin, which can be briefly summarised as follows: Stimulation of sensory neurons mediating pain reception in the airways triggers escape or homeostatic adaptation. Rats are able to adapt to environmental changes by reducing their energetic needs through a reversible state of suppressed metabolic demand and reduced body temperature ('hibernation-like state'). Under such conditions, the delivery of oxygen to the tissues is reduced and this reduction is counterbalanced by decreased tissue oxygen demand at lower temperatures. However, when this occurs in pregnant rats, the altered oxygen delivery to the (rapidly growing) foetus may have developmental consequences. A more detailed description of this MoA can be found in the publication by Pauluhn (2018).

The foetal hypoxia in the current study could have been at least partly compensated by the two-fold increase in partial pressure of oxygen in the oxygen-supplemented group. The reduced incidence of eye malformations in the oxygen-supplemented group (from 14 to 7 foetuses, from 9 to 5 litters) indicates that foetal hypoxia did play a role in their aetiology. On the other hand, the incidence of malformations did not drop to control levels. Nevertheless, it is noted that oxygen supplementation did not fully counteract the altered metabolic status of maternal animals; at least hypothermia was still present also in the oxygen-supplemented group.

RAC further notes that microphthalmia was always present in concurrent controls and that no increase in microphthalmia was observed in oral PNDT studies up to maternally toxic doses associated with plasma levels markedly (at least 10-fold) higher than in the inhalation studies (for details see 'Supplemental information in the Background document').

Taking into account all available information, RAC considers maternal adaptive mechanisms triggered by sensory irritation as a plausible MoA behind the increased incidence of microphthalmia in studies 77 and 78, although there are some remaining uncertainties (e.g. the fact that oxygen supplementation did not completely prevent an increase in microphthalmia).

Based on the available evidence, RAC considers plausible that the increased incidence of microphthalmia in the rat inhalation studies 77 and 78 resulted from maternal adaptive mechanisms ('hibernation-like state' involving bradypnoea and hypothermia) triggered by sensory irritation. As this strong physiological response observed in rats is not likely to be tolerated by humans exposed to (beta-)cyfluthrin, the increase in eye malformations is considered of low human relevance.

Increased post-implantation loss in one of the rabbit studies (study 75) could be considered borderline for classification in Category 2. However, taking into account the magnitude of the increase, and concomitant maternal toxicity, RAC concluded that this effect is not sufficient to trigger classification.

Overall, **RAC agrees with the DS's proposal of no classification for developmental toxicity**.

Adverse effects on or via lactation

Findings in the offspring attributable to effects on or via lactation were observed in two studies: in the two-generation study with cyfluthrin (study 70; tremors, reduced pup body weight by up to 25%) and in the DNT study with beta-cyfluthrin (study 80; reduced pup body weight by ca. 10%). The magnitude of pup weight reduction in the DNT study is not considered sufficient for classification. Therefore, the assessment will be focused on the two-generation study.

Two-generation study in rats with cyfluthrin (study 70)

Cyfluthrin was administered at dietary concentrations of 0, 50, 125 and 400 ppm. Coarse tremors were observed in mid-and high dose pups from PND 5 to 18. Pup body weight reduction on PND 7 reached 25% at the top dose; the effect at the mid-dose was weaker (11%). Findings at the top dose are considered less relevant for classification due to concurrent maternal neurotoxicity (splayed hind limbs). However, the incidence of coarse tremors at the mid-dose without maternal toxicity is still rather high especially in the F2 generation.

Two-generation study in rats (study 70): effects during lactation				
Dose (ppm)	**0**	**50**	**125**	**400**
Dose (mg/kg bw/d) during lactation	0	7	19	59
F0/F1				
Incidence of splayed hind limbs in dams	0/30	0/27	0/26	15/29*
Litter incidence of coarse tremors in pups; [day of onset - day of last occurrence]	0/30	0/27	4/25 [PND 7–15]	15/28* [PND 5–17]

Pup bw on PND 1, males + females (g)	6.6	6.6	6.4	6.6
Pup bw on PND 4 post-culling (g)	10.0	10.3	9.7	9.2* (-8%)
Pup bw on PND 7 (g)	16.2	16.4	15.0* (-7%)	13.7* (-15%)
Pup bw on PND 14 (g)	31.4	31.5	29.5* (-6%)	25.2* (-20%)
Pup bw on PND 21 (g)	49.0	50.1	46.1	39.4* (-20%)
F1/F2				
Incidence of splayed hind limbs in dams	0/25	0/27	0/27	9/25*
Litter incidence of coarse tremors in pups; [day of onset - day of last occurrence]	0/25	0/26	19/26* [PND 7–16]	9/25* [PND 7–13]
Pup bw on PND1 (g)	6.7	6.4*	6.4	6.3* (-6%)
Pup bw on PND 4 post-culling (g)	10.3	9.3*	9.5	8.2* (-20%)
Pup bw on PND 7 (g)	16.1	14.7*	14.4* (-11%)	12.0* (-25%)
Pup bw on PND 14 (g)	30.3	28.8	25.8* (-15%)	23.0* (-24%)
Pup bw on PND 21 (g)	45.4	42.8	39.0* (-14%)	33.6* (-26%)

* Statistically significant difference from control, $p \leq 0.05$

Although cyfluthrin levels in milk were not measured in this study, occurrence of neurotoxicity in pups as early as PND 5 (*i.e.* before pups start feeding on maternal diet) strongly indicates transfer via milk. The DNT study with beta-cyfluthrin (study 80) reported a concentration-dependent increase in test substance concentration in foetal brains already on PND 4, which again indicates a transfer of the substance via milk. No neurotoxic symptoms were observed in the DNT study up to 200 ppm.

Transfer of the substance into milk was confirmed in lactating cows and goats. The parent substance was the major residue in cow and goat milk (EFSA, 2018). Cyfluthrin was also detected in human breast milk samples in several studies (e.g. Bouwman *et al.*, 2006; Feo *et al.*, 2012).

According to CLP, classification for effects on or via lactation can be assigned based on results of one- or two-generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk.

Coarse tremors in pups in the two-generation study with cyfluthrin, although transient, are considered an adverse effect. At 125 ppm the tremors occurred in the absence of maternal toxicity. The weight of evidence (high lipophilicity, tremors began before the pups started to feed on maternal diet, substance was detected in pup brains on PND 4 in the DNT study, transfer via milk documented for cows, goats and humans) is sufficient to establish that the tremors are a consequence of transfer in the milk.

Industry proposed no classification based on lack of human relevance, assuming that breastfed babies would not be more susceptible to neurotoxicity of (beta-)cyfluthrin than their mothers. While RAC agrees that lower metabolic capacity of neonatal rats compared to adult animals is a plausible explanation of their increased susceptibility, the available

data do not indicate that the situation in humans should be different from that in rats at least for infants under three weeks of age (see 'Supplemental information in the Background document').

Industry further argued that humans (including lactating females) would never be exposed to the high concentrations of (beta-)cyfluthrin required to overwhelm the metabolising capacity of the sensitive neonate rat. Nevertheless, risk-based arguments cannot be taken into account in hazard assessment.

Thus, **RAC agrees with the DS's proposal to classify for Lact.; H362** mainly based on coarse tremors in pups of the two-generation study (study 70) attributable to transfer of the test substance via milk and occurring in the absence of maternal toxicity.

Supplemental information - In depth analyses by RAC

Comparison of plasma levels after oral and inhalation exposure

The available information on plasma levels of cyfluthrin or beta-cyfluthrin (parent substance) in rats after oral and inhalation exposure is summarised in the following table.

Plasma concentration of cyfluthrin or beta-cyfluthrin (parent substance) after oral and inhalation exposure

Type of study	Method	Concentration in plasma	Reference
Oral absorption after single administration	Rat, Wistar (BOR:WISW), males Single dose of 10 mg/kg bw, gavage, cyfluthrin (non-radiolabelled) Vehicle Cremophor EL/water or PEG 400 Sacrifice at 0.5, 1, 2, 4, 6, 16, 24 h after administration 2 animals per vehicle and time point of sacrifice Cyfluthrin determined in the blood and in stomach extracts	Cremophor EL/water: T_{max} 1 h C_{max} 0.30 µg/mL (blood) PEG 400: T_{max} 6 h C_{max} 0.075 µg/mL (blood) Note: plasma levels assumed to be approx. 2-fold higher than blood levels (based on the results of studies 85 and 87)	Study 88
Toxicokinetics after single oral and intravenous administration	Rat, Wistar, males Oral part: Single dose of 20 mg/kg bw, gavage, cyfluthrin (non-radiolabelled) Vehicle corn oil Sacrifice at 0.16, 0.33, 0.5, 1, 2, 4, 6, 8, 12 and 24 h after administration 8 animals per time point of sacrifice	Corn oil: T_{max} 3.4 h C_{max} 0.39 µg/mL	Rodríguez *et al.* (2018)

	Cyfluthrin determined in the plasma and in the brain by LC-MS		
ADME, single oral administration	Rat, Wistar, males Single dose of 10 mg/kg bw, gavage, beta-cyfluthrin (radiolabelled on the fluorophenyl) Vehicle PEG 400 4 animals per group	PEG 400, sampling time 6 h: Beta-cyfluthrin 0.15 µg eq./g The remaining extractable fractions 9.61 µg eq./g, non-extractable 0.89 µg eq./g	Study 87
PNDT study, inhalation	Rat, Wistar (BOR:WISW), pregnant females Toxicokinetic part: Exposure GD 6-13, 6 h/d Concentrations 0 (air), 0 (vehicle), 0.46, 2.55, 11.9, 12.8+O_2 mg/m^3, cyfluthrin Vehicle ethanol/PEG 400 5 animals per concentration Cyfluthrin determined in the plasma immediately post-exposure (GD 13)	At 11.9 mg/m^3: mean 19.0 pmol/mL = 8.3 ng/mL range (8.5–38.5) pmol/mL = (3.7–17) ng/mL At 12.8 mg/m^3+O_2: mean 14.7 pmol/mL = 6.1 ng/mL range (9.2–18.3) pmol/mL = (4.0–7.9) ng/mL Recovery rate from plasma ca. 30–60%, no correction for recovery made	Study 78/79

There are two key oral PNDT studies in the rat (studies 72 and 76), both negative. The top doses in these PNDT studies were 30 or 40 mg/kg bw/d, Cremophor or PEG 400 were employed as vehicles. The C_{max} (plasma) values for the parent substance in oral toxicokinetic studies ranged from 0.15 µg/mL (PEG 400) to ca. 0.6 µg/mL (Cremophor); these plasma levels relate to an administered dose of 10 mg/kg bw, which is 3 to 4 times lower than the top doses in the PNDT studies. In comparison, plasma levels of the parent substance at a concentration causing microphthalmia in the inhalation PNDT study 78 ranged from ca. 0.008 to 0.04 µg/mL (after correction for incomplete recovery). Based on this information, systemic exposure to the parent substance was markedly (at least 10-fold) higher in the negative oral PNDT studies than in the positive inhalation PNDT studies.

RAC notes that the oral toxicokinetic studies were conducted in males while developmental toxicity relates to a female situation. The higher sensitivity of males compared to females in some acute toxicity studies suggests some toxicokinetic or toxicodynamic difference. Nevertheless, the sex difference in C_{max} is expected to be rather small given that a ca. 50-fold difference in LD_{50} between studies with Cremophor vs. PEG 400 corresponds to a ca. four-fold difference in C_{max} (study 88), and the difference between female and male LD_{50} values was two-fold or less.

Cyfluthrin and beta-cyfluthrin are extensively metabolised upon oral exposure. According to study 87, only about 1.5% of the radiolabel in the plasma is the parent substance, the rest are metabolites. The metabolism after inhalation exposure is not expected to be significantly higher than after oral exposure. Consequently, the difference between plasma

levels after oral vs. inhalation exposure found for the parent substance is considered to apply also to the parent substance plus metabolites.

Rabbit PNDT study 75: relationship between maternal food consumption and post-implantation loss

The table below shows that there was no strong correlation between post-implantation loss and maternal food consumption during the treatment period at the top dose of 180 mg/kg bw/d.

Post-implantation loss and food consumption at 180 mg/kg bw/d in study 75

Dam no.	Food consumption GD 6-19 (g/animal/d)	Post-implantation loss (%; absolute numbers)	Embryonic resorptions (%; absolute numbers)
49	34	0 (0/15)	0 (0/15)
50	25	53 (9/17)	29 (5/17)
51	93	13 (1/8)	13 (1/8)
52	50	37 (7/19)	32 (6/19)
53	63	0 (0/12)	0 (0/12)
54	71	100 (9/9)	100 (9/9)
55	77	18 (2/11)	0 (0/11)
56	38	75 (9/12)	75 (9/12)
57	87	60 (6/10)	50 (5/10)
58	123	0 (0/9)	0 (0/9)
59	146	31 (5/16)	7 (1/16)
60	114	38 (6/16)	7 (1/16)
61	97	14 (1/7)	0 (0/7)
62	52	20 (2/10)	10 (1/10)
63	98	27 (4/15)	27 (4/15)
64	45	11 (1/9)	11 (1/9)
Mean	76	31	22
Control mean	131	11	4

Human relevance of tremors in rat sucklings

Neonatal rats are more sensitive than adults to cyfluthrin-induced neurotoxicity, presumably due to lower metabolic capacity (US EPA, 2010; Anand *et al.*, 2006). Industry proposed that this age dependence of metabolic capacity towards pyrethroids does not exist in humans.

The major contributors to pyrethroid metabolism in humans are P450 enzymes CYP2C8, CYP2C19 and CYP3A4 together with carboxylesterhydrolases CES1 and CES2 (Song *et al.*, 2017). In general, for many drug metabolising enzymes a substantial increase in expression is observed within the first one or two years after birth. This seems to be the

case for CYP3A4 while the increase for CYP2C19 is rather moderate (Hines, 2008). A steep increase of CYP2C8 content in human microsomes was found to occur around postnatal day 35 (Song *et al.*, 2017). Hines *et al.* (2016) reported an increase in human microsomal and cytosolic CES 1 and CES 2 around three weeks of age. Overall, these data indicate that human infants younger than three weeks would exhibit significantly lower pyrethroid clearance compared with adults.

4.10.6.1 Neurotoxicity

Hazard class not assessed in this dossier

4.10.6.2 Immunotoxicity

Hazard class not assessed in this dossier

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

Table 83: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Aqueous hydrolysis at pH 4, 7 and 9 cyfluthrin and beta-cyfluthrin, 4 µg L^{-1} (sum of all diastereomers) OECD Guideline No. 111	pH 4: hydrolytically stable, half-live > 1 year at 20 °C (all isomers) pH 7: half-live 270 days (isomers I + II) and 160 days (isomers III + IV) pH 9: half-live 42 hours (isomers I + II) and 33 hours (isomers III + IV)	Due to epimerization both test substances formed mixtures of identical composition under the conditions of this test. half-lives for 20 °C were calculated by extrapolation	Krohn (1997) Xu & Ripperger (2013)
Photo degradation in sterile water at pH 7 beta-cyfluthrin OECD Guideline No. 101	Half live : 5.2 – 56 days Mean quantum yield Φ of 0.001149	moderate photo-degradation of beta-cyfluthrin	Hellpointner & Malburg (2013)
Ready biodegradation	No data		
Biodegradation in water/sediment systems beta-cyfluthrin SETAC (1995) & German BBA Part IV, 5-1 (aerobic at 20 ± 1°C in the dark, 100 days)	DT_{50} = 14.4 – 53 days (20°C) total system DT_{50} = 14.3 – 81.5 days (20°C) sediment	Two systems tested (small disused gravel pit, catchment basin)	Sneikus (2000) Hammel & Porschewski (2013)

5.1.1 Stability

Hydrolytic degradation

Reference	:	Xu, Tianbo and Ripperger, Randy
Title	:	Cyfluthrin and beta-cyfluthrin – hydrolysis half-live evaluation
Year of execution	:	14 June 2013
GLP statement	:	not relevant
Guideline	:	not relevant; calculation according OECD 111
Test substance	:	Cyfluthrin and beta-Cyfluthrin
Test system	:	hydrolysis under sterile conditions

Executive Summary

The original study (Krohn, 1997) was re-evaluated and the detailed half-lives were calculated according OECD guideline 111. The re-evaluation showed that the half-lives reported in the original study report (Krohn, 1997) were calculated according the OECD 111.

Study design

The half-lives from the original study of Krohn (1997) were re-evaluated. No experiment was performed for the re-evaluation.

Description

According to OECD guideline no. 111 (Annex 2), the Arrhenius equation can be used to calculate the rate constant k for other temperatures, when the rate constants are known for 2 temperatures. The linear relationship between rate constants at higher temperatures and reciprocals of temperature in Kelvin were calculated. Afterwards, the rates at lower temperatures were extrapolated by using the linear regression equations. These rates were used to calculate the half-lives.

Results

The half-lives recalculated by Xu & Ripperger in comparison to those by Krohn 1997 are summarized in Table below. Only data for 20 °C are given here.

As can be seen in Table 66, the half-lives in the original study report were the same as the half-lives calculated according to OECD guidelines with three exceptions as highlighted. However, the differences were very small (4750 *versus* 4740 hours and 41.5 *versus* 41.6 hours) and assumed to be caused by the rounding during the calculations.

Conclusions

The half-life values in the original study report were the same as the half-life values calculated according to OECD guidelines.

Table 84: Comparison of half-lives recalculated by Xu & Ripperger 2013 with data from Krohn 1997

pH	Test substance	Isomers	Temperature °C	half-lives recalculated (hours)	half-lives in original study (hours)
7.0	cyfluthrin	isomers I + II	20	6560	6560
		isomers III + IV	20	4750	4740
	beta-cyfluthrin	isomers I + II	20	6610	6610
		isomers III + IV	20	3060	3060
9.0	cyfluthrin	isomers I + II	20	36.6	36.6
		isomers III + IV	20	24.5	24.5
	beta-cyfluthrin	isomers I + II	20	46.5	46.5
		isomers III + IV	20	41.5	41.6

Photochemical degradation

Reference	:	Hellpointner, E. and Malburg, G.
Title	:	Beta-cyfluthrin: determination of the quantum yield and assessment of the environmental half-live of the direct photo-degradation in water
Year of execution	:	02 September 2013
GLP statement	:	Yes
Guideline	:	OECD test guidelines 101, 1981 and 316, 2008
Test substance	:	beta-Cyfluthrin
Test system	:	photolysis in water

Executive Summary

The UV-VIS absorption spectrum of beta-cyfluthrin was determined in water/acetonitrile (50/50, v/v). It showed one weak maximum at 268 nm (ε = 2082 L × mol-1 cm-1, bandwidth up to 281 nm). In buffered aqueous solutions of pH 4 and pH 7 the UV-VIS absorption properties were similar. The molar extinction coefficients ε at 290 and 295 nm were determined to be 158 and 84 L × mol^{-1} cm^{-1}, respectively. The absorption of beta-cyfluthrin extends only weak but very far into the environmentally relevant range of wavelengths. This indicates some potential for direct photolytic interactions of beta-cyfluthrin with sunlight.

The quantum yield of direct photo-transformation of the beta-cyfluthrin was determined in above mentioned aqueous solution using polychromatic light according to the ECETOC method. By HPLC analysis a beta-cyfluthrin degradation of 18.5% - 20.9% was measured after a maximum irradiation period of 500 minutes. This indicates a moderate degradability of beta-cyfluthrin via direct photo-transformation in aqueous solutions. A low mean quantum yield (Φ) of 0.001149 was calculated on the basis of UV absorption data and the kinetics determined from two degradation experiments.

A comparison of the estimates derived from models of Zepp & Cline and Frank & Kloepffer shows that both approaches are well comparable. The two approaches consider the quantum yield and the absorption in a range of wavelengths relevant for the environment. Environmental half-lives in top surface water layer exposed to sunlight were estimated to range between one week and one month, for direct phototransformation of beta-cyfluthrin during periods of main use in May and June. Direct photo-transformation in water may contribute to the dissipation of beta-cyfluthrin from the environment.

Material and methods

Test material

Non-radiolabelled beta-cyfluthrin, purity 98.8 %, minimum concentration of diastereomers II + IV is 95 % in the pure active substance, 30 – 42 % cis / 58 – 70 % trans.

Study design

UV-VIS absorption spectra

For the determination of UV-VIS spectra of beta-cyfluthrin, a stock solution of beta-cyfluthrin was prepared in acetonitrile (ACN). Buffered aqueous solution of pH 4 and pH 7 were prepared by diluting the required aliquots of stock solution with the respective 0.01 M aqueous acetate (pH 4) or 0.01 M phosphate (pH 7) buffer solutions. In addition, a solution of beta-cyfluthrin in water/ACN (1:1, v:v) was prepared (15.41 mg beta-cyfluthrin L-1) and used for calculating the quantum yield and for the corresponding environmental modelling.

The UV-VIS absorption properties of the test item were characterised by the number and position of the absorption maxima as well as for each maximum by the molar extinction coefficient ε [L × mol-1 cm-1]. Moreover, the bandwidth was determined for each resolved maximum. In case of the respective UV-VIS spectrum, the extinction values were considered until 490 nm.

Quantum yield

The experiment was based on the method by ECETOC. It is based on the partition of the polychromatic light into sectors of wavelengths. In this study, sectors of 5 nm were defined 295 – 400 nm and sectors of 10 nm from 401 nm on.

Photo-transformation

For the solutions for the photo-transformation experiments, 0.195 mL of the beta-cyfluthrin stock solution were pipetted into a 50-mL volumetric flask and made up to volume with water (c = 5.01 mg/L) corresponding to 1.15 × 10-5 mol L-1. The degradation experiment was conducted in a merry-go-round irradiation apparatus which was fitted with a mercury immersion lamp TQ 150. The light intensity acting on the test solution was measured by means of uranyl oxalate as chemical actinometer and the total amount of radiation entering the measuring cell was calculated from the number of photons being absorbed by the actinometer. From the titration difference derived from actinometry, the average intensity of radiation acting upon the test solution during the exposure was calculated.

The results of beta-cyfluthrin analysis from the photo-transformation test (usually means of duplicates) were evaluated on the basis of linear regression and represented as a degradation line in a concentration versus time diagram (log % beta-cyfluthrin = - kt + b). The time (in min) after which 10% of the molecules of the test item have been degraded (necessary to calculate the quantum yield) was calculated according to the decay law from the determined rate constant k [1/min] of photo-transformation (single first order degradation).

Description of analytical procedures

The beta-cyfluthrin concentration in the liquid samples was determined by reversed phase HPLC and evaluation of the respective UV signals (as sum of the main two diastereomeric enantiomer pairs, II + IV) by means of external reference standard). A significant change of ratio of diastereomer pairs (II : IV) was not measured during the irradiation.

Results

UV-VIS absorption spectra:

The UV-VIS absorption spectrum of a solution of 15.41 mg beta-cyfluthrin/L water/ACN (1/1, v/v) showed one weak maximum at 268 nm (abs 0.0739, ε 2082 L × mol^{-1} cm^{-1}). The respective UV-VIS absorption spectra of 15.41 mg beta-cyfluthrin per litre pH 4 and pH 7 buffered aqueous solutions showed similar absorption properties. The molar extinction coefficient ε at 290 and 295 nm was determined to be 158 and 84 L × mol^{-1} cm^{-1}, respectively. In general, the absorption properties indicate a weak potential for direct photolytic interactions of beta-cyfluthrin with sunlight in the environment.

Photo-transformation – Intensity of irradiation

The intensity of irradiation was calculated to 7.8482 – 7.8025 x 10^{16} photons absorbed per second for the 3 mL actinometry solution in the range of wavelength from 295 to 490 nm. By HPLC- analysis a beta-cyfluthrin degradation of 18.5% - 20.9% was measured after a maximum irradiation period of 500 minutes. This indicates a moderate degradability of beta-cyfluthrin via direct photo-transformation in aqueous solutions.

Photo-transformation – Quantum yield

Based on both degradation experiments performed, quantum yields Φ of 1.0980 – 1.2005 × 10^{-3} were calculated. Thus, a mean quantum yield Φ of 0.001149 was obtained for the direct photo-transformation in aqueous solution.

Photo-transformation – Environmental half-lives according to Zepp & Cline

Environmental half-lives were calculated according to Zepp & Cline (GC Solar) by using an arithmetic model which allows for a transfer of laboratory data concerning the direct photo-transformation in water to field conditions. Based on a mean quantum yield Φ of 0.001149 and the molar extinction coefficients ε from 297.5 to 490 nm, environmental half-lives were calculated and summarised in Table 60.

Table 85: Environmental half-lives (days) calculated according to Zepp &Cline

Season	Degree of latitude			
	30°	40°	50°	60°
Spring	5.51	5.88	6.57	7.76
Summer	4.97	4.99	5.14	5.44
Fall	7.65	9.71	14.1	25.9
Winter	10.2	15.4	30.2	93.5

Marginal conditions: pure surface water at 0-5 cm depth, 10th degree longitude, clear sky, typical ozone concentrations in the atmosphere, half-lives integrated over the entire day. The results for the 50th degree of latitude are regarded to be relevant to the conditions of Central Europe.

Photo-transformation – Environmental half-lives according to Frank & Kloepffer
Environmental half-lives were also calculated according to Frank & Kloepffer, using an arithmetic model which considers the influence of clouded sky for the region Central Europe, i.e. Germany. Using the mean quantum yield Φ of 0.001149 and the molar extinction coefficients ε from 292.5 to 490 nm, environmental half-lives were calculated as summarized in Table 68.

Table 86: Environmental half-lives (days) calculated according to Frank & Kloepfer

Month	Minimum	Mean	Maximum
April	6.1	11	44
May	5.4	8.7	35
June	5.2	7.7	31
July	5.8	8.7	29
August	5.9	8.9	30
September	9.0	15	56

Marginal conditions: pure stagnant surface water at 0-5 cm depth, geographic and climatic conditions of Germany (50° lat.), no contribution of another mono- or bimolecular elimination process.

Conclusion
In general, the absorption properties indicate a weak potential for direct photolytic interactions of beta-cyfluthrin with sunlight in the environment. A moderate photo-degradation of beta-cyfluthrin in aqueous solution in a range of 19 to 21% was measured by HPLC after a maximum irradiation period of 500 minutes. A low mean quantum yield of Φ = 0.001149 was calculated on the basis of UV absorption data and the kinetics determined from two degradation experiments.

A comparison of the estimates derived from models of Zepp & Cline and Frank & Kloepffer shows that both approaches are well comparable. The two approaches consider the quantum yield and the absorption in a range of wavelengths relevant for the environment. Environmental half-lives in top surface water layer exposed to sunlight were estimated to range between one week and one month, for direct phototransformation of beta-cyfluthrin during periods of main use in May and June.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

5.1.2.2 Screening tests

No data available.

5.1.2.3 Simulation tests

Two water-sediment studies were carried out to investigate the route and rate of degradation or dissipation of beta-cyfluthrin with different radioactive labelling (Anderson 1987, Sneikus 2000). The main metabolites were FPB-aldehyd (16 % in sediment) and FPB-acid (29 % in water).

The studies were evaluated in the monograph dated 01 October 1996 and the addendum 1 dated 07 May 2002. However, the degradation rates were recalculated by Hammel & Porschewski, 2013 following the latest FOCUS kinetic guidelines.

Reference	:	Hammel, Klaus and Porschewski, Ruth
Title	:	Kinetic evaluation of the aerobic aquatic metabolism of cyfluthrin and beta-cyfluthrin and their metabolites in water/sediment systems according to FOCUS kinetics
Year of execution	:	26 November 2013
GLP statement	:	not relevant
Guideline	:	not relevant; calculation according FOCUS Kinetics (2006)
Test substance	:	Cyfluthrin and beta-Cyfluthrin
Test system	:	aerobic degradation in water/sediment systems under laboratory conditions

The water/sediment study of **Sneikus 2000** was evaluated in the addendum 1 to the monograph of October 1996, dated 07 May 2002. For overview of the test systems the data are given in Table below.

Table 87: Analytical data of water phases and sediments in the study of Sneikus 2000 (short summary)

	Barmener See (Jülich, DE)	**Genkel creek (Meinerzhagen, DE)**
	small disused gravel-pit	**catchment basin**
water phase during experiment		
O_2 content (% saturation)	57 - 124	49 - 114
pH	5.1 – 8.1	4.6 – 8.0
total org C (TOC, mg C L^{-1})	start: 2.7 / end: 15	start: 1.5 / end: 12
dissolved org C (DOC mg C L^{-1})	start: 2.4 / end: 4	start: 1.5 / end: 2
hardness (° dGH)	start: 8	start: 4
total N (mg L^{-1})	start: 1.95 / end: 10.0	start: 2.12 / end: <1
total P (mg L^{-1})	start: 0.1 / end: 0.36	start: 0.01 / end: 0.27
sediment at start of experiment		
sand (2000 – 63 µm) %	97.7	8.2
silt (63 – 2 µm) %	5.3	73.4
clay (< 2 µm) %	<0.1	18.4
Texture	sand	silt loam
organic C (%)	0.48	4.91
organic matter (OC × 1.72)		
total N (%)	0.18	0.43
total P (mg/kg)	119	835
$CaCO_3$ (g/kg)	0.5	<0.1
pH (H_2O / $CaCl_2$)	7.5 / 6.9	5.0 / 4.6

The test substance was ^{14}C-labelled (cyclopropane-1-^{14}-C)-cyfluthrin at a concentration of 8.14 µg/L.

The study was conducted at a temperature of 20 °C in the dark over a period of 100 days with samplings at 0.5, 3 and 6 hours and at day 1, 2, 3, 7, 10, 14, 28, 56 and 100. In the study of Sneikus 2000 the diastereomers were analysed separately and the ratio of the diastereomers was measured at the different sampling dates. To account for beta-cyfluthrin the relative amounts of the two active diastereomers II and IV were summed up and combined with the measured concentration (% AR) of cyfluthrin. The cyfluthrin residues in total systems are given in Table 70, together with relative amounts of isomers II + IV and beta-cyfluthrin residues. The residues for the metabolite DCVA in the water/sediment system are given in Table 71. The data for total system are used for kinetic analysis (decline fit from maximum).

Table 88: Cyfluthrin in water, sediment extract and total system and beta-cyfluthrin in total system from Sneikus 2000

Time	Cyfluthrin (% AR)						% Isomers II + IV		beta-Cyfluthrin (% AR)	
	Barmener			Genkel			Barmener	Genkel	Barmener	Genkel
	Water	Sediment	Total	Water	Sediment	Total			Total system	
0.5 h	46.94 +	41.91	92.43 *	29.71 +	63.80	98.14 *	47 #	43 #	39.01	41.42
0.5 h	46.29 +	41.95	91.45 *	28.15 +	58.31	90.58 *			38.59	38.22
3 h	17.74	64.58	82.32	37.12	42.18	79.30	47	41	38.36	37.35
3 h	31.91	56.01	87.92	25.98	55.69	81.67			40.97	38.47
6 h	22.54	68.36	90.90	17.53	66.37	83.90	42	44	40.91	37.76
6 h	NaN	NaN	NaN	18.35	59.65	78.00			NaN	35.10
1 d	15.88	48.18	64.06	3.34	56.05	59.39	48.7	38	31.20	22.57
1 d	18.69	48.25	66.94	4.64	57.22	61.86			32.60	23.51
2 d	3.38	35.15	38.53	1.14	52.49	53.63	48.8	38	18.80	20.38
2 d	2.65	46.77	49.42	1.00	62.82	63.82			24.12	24.25
3 d	0.32	39.98	40.30	0.61	49.09	49.70	40.8	37.1	16.44	18.44
3 d	0.35	41.86	42.21	0.68	44.03	44.71			17.22	16.59
7 d	LOD	25.07	25.11	0.05	42.74	42.79	40.4	38.9	10.14	16.65
7 d	LOD	25.69	25.69	0.16	38.45	38.61			10.38	15.02
10 d		21.75	22.41	0.31	31.06	31.37	31.6	36.1	7.08	11.32
10 d		22.12	22.82	2.15	28.93	31.08			7.21	11.22
14 d		15.15	15.15		41.80	41.80	46.8	34.4	7.09	14.38
14 d		10.98	10.98		32.61	32.61			5.14	11.22
28 d		9.33	9.33		19.25	19.25	49.5	33.6	4.62	6.47
28 d		13.32	13.32		19.02	19.02			6.59	6.39
56 d		7.30	7.30		16.49	16.49	36.2	41.4	2.64	6.83
56 d		7.87	7.87		18.21	18.21			2.85	7.54
100 d		7.01	7.01		13.30	13.30	38.8	38.1	2.72	5.07
100 d		7.17	7.17		18.45	18.45			2.78	7.03

* total recocery of radioactivity in whole system
+ including dissolved ^{14}C-carbonates
NaN : not a number, only single measurement at 6 h
LOD : limit of detection, 0.05 %
relative amounts before application (day 0) is 42.2 % for both systems Barmener and Genkel

Table 89: Metabolite DCVA in water, sediment and total system from study by Sneikus 2000

Time	Barmener			Genkel		
	Water	Sediment	Total	Water	Sediment	Total
0.5 h			0.00			0.00
0.5 h			0.00			0.00
3 h	4.01		4.01	3.44		3.44
3 h	3.83		3.83	3.49		3.49
6 h	5.45	0.28	5.73	7.37	0.45	7.82
6 h	NaN	NaN	0.00	8.30	0.25	8.55
1 d	19.63	0.76	20.39	17.13	3.18	20.31
1 d	16.03	0.50	16.53	18.47	2.46	20.93
2 d	39.81	4.45	44.26	17.88	4.41	22.29
2 d	32.15	4.44	36.59	11.97	2.56	14.53
3 d	21.00	1.73	22.73	23.34	5.27	28.61
3 d	21.90	1.41	23.31	21.88	6.39	28.27
7 d	32.83	4.69	37.52	24.53	9.14	33.67
7 d	23.61	1.21	24.82	23.69	9.56	33.25
10 d	37.33	5.53	42.86	21.51	10.80	32.31
10 d	26.67	4.00	30.67	26.77	11.11	37.88
14 d	20.55	4.19	24.74	21.04	8.04	29.08
14 d	11.61	1.57	13.18	24.50	10.36	34.86
28 d	20.46	2.29	22.75	31.76	16.35	48.11
28 d	32.29	3.43	35.72	32.60	14.55	47.15
56 d	34.03	4.73	38.76	28.51	17.37	45.88
56 d	30.85	5.08	35.93	28.97	18.64	47.61
100 d	19.52	7.71	27.23	7.83	20.37	28.20
100 d	31.76	8.28	40.04	14.46	26.93	41.39

Modelling

The water/sediment studies were evaluated by Hammel & Porschewski 2013 according to FOCUS 2006. The dissipation or degradation kinetics are evaluated only in single compartments (level P-I and M-1). The model fit and the statistical evaluation of the results was carried out with the software KinGui version 2, developed by Bayer Crop Science. For the optimisation of the algorithms, Iteratively Reweighted Nonlinear Least Squares (IRLS) was used.

Results

The relevant criteria to select the appropriate model which describes the residues of beta-cyfluthrin in the total systems are given in Table 72.

Table 90: Criteria to select models for beta-cyfluthrin in total systems

	Model	chi^2 (%)	Visual fit	t-test p-value	Selected
Barmer	SFO	13.06	-	< 0.001	
Genkel		21.08	-	0.493	
Barmer	HS	5.45	+	0.004 (k_2)	
Genkel		11.15	o	0.001 (k_2)	
Barmer	FOMC	7.03	o	na	
Genkel		7.45	+	na	trigger
Barmer	DFOP	5.03	+	0.009	mod.+trigger
Genkel		9.83	+	0.010	modelling

visual fit: + good, o acceptable, - not acceptable
na: t-test not applicable for FOMC alpha or beta

The relevant criteria to select the appropriate model which describes the residues of beta-cyfluthrin in the sediment are given in Table 73.

Table 91: Criteria to select models for beta-cyfluthrin in sediment

	Model	chi² (%)	Visual fit	t-test p-value	Selected
Barmer	SFO	16.50	-	<0.001	
Genkel		18.62	-	0.003	
Barmer	HS	8.02	-	0.006 (k_2)	
Genkel		11.84	o	0.022 (k_2)	modelling
Barmer	FOMC	5.43	+	na	mod.+trigger
Genkel		8.44	+	na	trigger
Barmer	DFOP	5.63	+	0.015 (k_2)	
Genkel		10.46	o	0.161 (k_2)	

visual fit: + good, o acceptable, - not acceptable
na: t-test not applicable for FOMC alpha or beta

The resulting data for DT_{50} and DT_{90} of beta-cyfluthrin are given in Table 74 and 75 both for triggers and for modelling endpoints.

Table 92: Kinetic parameters for degradation of beta-cyfluthrin in total system

System	Trigger endpoints			Modelling endpoints				
	Model	DT_{50} days	DT_{90} days				DT_{50}	DT_{90}
beta-cyfluthrin								
Barmener	DFOP	2.4	47.9	DFOP	k_1 0.3826 k_2 0.0128 g 0.815		14.4 *	47.9
Genkel	FOMC	2.4	295.4	DFOP	k_1 0.6929 k_2 0.0131 g 0.613		53.0 (k_2)	103.7
geometric mean		2.4					27.6	

* DT90 / 3.32, backcalculation as final residues < 10 % of applied

Table 93: Kinetic parameters for dissipation of beta-cyfluthrin in sediment

System	Trigger endpoints			Modelling endpoints				
	Model	DT_{50} days	DT_{90} days	Model			DT_{50} days	DT_{90} days
beta-cyfluthrin								
Barmener	FOMC	3.1	47.6	FOMC			14.3 *	47.6
Genkel	FOMC	6.9	650.8	HS	k_1 0.0890 k_2 0.0085		81.5 (k_2)	180.8
geometric mean		4.6					34.1	

* DT90 / 3.32, backcalculation as final residues < 10 % of applied

Conclusion

Degradation and dissipation rates for total system and sediment of the two systems are acceptable. The DT_{50} were 14.4 to 53.0 days (DT_{90} 47.9 to 103.7 days).

5.1.3 Summary and discussion of degradation

There is no study on ready biodegradability for beta-cyfluthrin available.

In water/sediment systems it was shown that beta-cyfluthrin was not rapidly degradable with DT_{50} values of 14.4 – 53.0 days (total system) and DT_{50} values of 14.3 – 81.5 days (sediment).

Beta-cyfluthrin is hydrolytically stable under acidic and neutral conditions. Aquatic photolysis is not considered to be an important transformation route for beta-cyfluthrin in the environment with DT_{50} of 5 - 56 days.

The results of the test on the biodegradation of beta-cyfluthrin in the water/sediment system and abiotic degradation show that beta-cyfluthrin is considered not rapidly degradable (a degradation < 70 % within 28 days) for purposes of classification and labelling.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

There are no data for beta-cyfluthrin available. Since read-across of cyfluthrin and beta-cyfluthrin is justified the data of cyfluthrin presented below.

Table 94: Adsorption/desorption – cyfluthrin

Method /Guideline	Tested Soils	Adsor-bed a.s.	K_a[1]	K_{aOC}[2]	K_d[3]	K_{dOC}[4]	K_a / K_d[5]	Degradation products		Reference
								Name	[%] of a.s.	
		[%]	[L.kg^{-1}]	[L.kg^{-1}]	[L.kg^{-1}]	[L.kg^{-1}]				
OECD 106 and US EPA 163-1										Burhenne, J. (1996)
Soil 1	Laacher Hof	85.6	1116	124000	1448	160889	0.77			
Soil 2	Borstel	84.2	1244	180290	974	141159	1.28			
Soil 3	Howe	88.1	1321	117946	1307	116696	1.01			
Soil 4	Sable91	88.4	1793	73484	1705	69877	1.05			

[1] K_a = Adsorption coefficient
[2] K_{aOC} = Adsorption coefficient based on organic carbon content
[3] K_d = Desorption coefficient
[4] K_{dOC} = Desorption coefficient based on organic carbon content
[5] K_a / K_d = Adsorption / Desorption distribution coefficient

Based on the adsorption/desorption study, cyfluthrin could be considered as being immobile in soil. The substance is strongly adsorbed to the soil (arithmetic mean K_{oc} of 4 soils: 123930 L.kg^{-1}). cyfluthrin as well as the distribution of isomers of cyfluthrin (diastereoisomers I-IV) remained unchanged in soil.

Table 95: Adsorption/desorption – metabolite DCVA

Method /Guideline	Tested Soils	pH H_2O	Ad-sorbed a.s.	K_a[1]	K_{aOC}[2]	K_d[3]	K_{dOC}[4]	K_a / K_d[5]	Degradation products		Reference
									Name	[%] of a.s.	
			[%]	[L.kg^{-1}]	[L.kg^{-1}]	[L.kg^{-1}]	[L.kg^{-1}]				
OECD 106 and US EPA 163-1											Slangen, P.M. (1999)
Soil 1	Speyer 2.1	6.9	19	0.184	31.0	0.676	114.2	0.27			
Soil 2	Cranfield 115	8.1	17	0.224	13.9	0.498	31.1	0.45			
Soil 3	Cranfield 230	5.1	77	2.893	356.2	5.678	699.2	0.51			

[1] K_a = Adsorption coefficient
[2] K_{aOC} = Adsorption coefficient based on organic carbon content
[3] K_d = Desorption coefficient
[4] K_{dOC} = Desorption coefficient based on organic carbon content
[5] K_a / K_d = Adsorption / Desorption distribution coefficient

Adsorption of DCVA depends on pH of the soils: leading to higher K_{oc} in acid soils. The metabolite DCVA (permethric acid) was considered as being mobile in soils Speyer 2.1 and Cranfield 115 and moderately mobile in soil Cranfield 230. The arithmetic mean K_{oc} of 3 soils is 133.7 L.kg^{-1} leading to a classification for DCVA to be mobile in soil. DCVA was stable during the adsorption/desorption study.

Table 78: Adsorption/desorption – metabolite FPB-acid

Method /Guideline	Tested Soils	pH H_2O	Adsor-bed a.s.	K_a[1]	K_{aOC}[2]	K_d[3]	K_{dOC}[4]	K_a / K_d[5]	Degradation products		Reference
									Name	[%] of a.s.	
			[%]	[L.kg^{-1}]	[L.kg^{-1}]	[L.kg^{-1}]	[L.kg^{-1}]				
OECD 106 and US EPA 163-1											Oddy, A. and Brett, R. (2005)
Soil 1	Pikeville	6.1	23-54	1.23	123	2.32	232	0.53			
Soil 2	Stanley	6.4	23-72	1.80	86	2.13	101	0.85			
Soil 3	Hofchen	7.2	27-78	1.03	50	1.22	59	0.84			
Soil 4	Laacher Hof	6.8	22-54	0.65	39	0.89	54	0.73			
Soil 5	Wurm-wiese	6.4	31-82	1.39	67	1.76	85	0.79			

[1] K_a = Adsorption coefficient
[2] K_{aOC} = Adsorption coefficient based on organic carbon content
[3] K_d = Desorption coefficient
[4] K_{dOC} = Desorption coefficient based on organic carbon content
[5] K_a / K_d = Adsorption / Desorption distribution coefficient

FPB-acid was found to be mobile (arithmetic mean K_{oc} of 5 soils: 73 L.kg^{-1} with marginal indication of pH-dependence). Due to limited duration of the adsorption period, the determined K_{OC} are shifted

to lower values. The compound was stable during the adsorption/desorption study in the limit of 5 hours investigation duration.

5.2.2 Volatilisation

The vapour pressure of the active isomers II and IV of beta-cyfluthrin ranges from 4.5 x 10^{-7} to 2.2 x 10^{-6} Pa at 20°C, direct evaporation is not expected, consequently. The Henry's Law Constants between 9.3 x 10^{-2} and 0.6 Pa × m^3 mol^{-1} at 20°C point to potential of volatility from water. On the other hand, the strong tendency to soil partition minimizes atmospheric entry.

The chemical lifetime of beta-cyfluthrin in the troposphere was calculated to be below one day (17.8 hours) using the Atkinson approach. Gathering from these results, accumulation of beta-cyfluthrin in the air is not to be expected.

5.3 Aquatic Bioaccumulation

Table 79: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
OECD 305, 28-day beta-cyfluthrin Flow-through; *Lepomis macrochirus*	BCF kin = 1822 [L kg^{-1}] Depuration: DT_{50} = 8.66 d	Because of malfunction in the flow system no reliable steady state in uptake phase was reached (BCF ss is not useful)	Anonymous (2014) Rep. No. D78913

5.3.1 Aquatic bioaccumulation

The log K_{ow} values of the isomers contained in beta-Cyfluthrin are greater than 3, i.e. 5.9 for isomer II and 5.8 for isomer IV (at 25°C). Therefore a bioconcentration study in fish is required.

5.3.1.1 Bioaccumulation estimation

5.3.1.2 Measured bioaccumulation data

Author:	Anonymous-B1
Title:	[Fluorophenyl-14C]Beta-Cyfluthrin: Bioconcentration Test in the Bluegill Sunfish (Lepomis Macrochirus) under Flow-Through Conditions
Date:	3 March 2014
Report no.:	D78913
Guidelines:	OECD Guideline No. 305
GLP:	yes
Validity:	valid

Executive Summary

The bioconcentration and depuration characteristics of beta-Cyfluthrin were investigated in the Bluegill sunfish in a dynamic flow-through system. The bioconcentration in whole fish was calculated.
The fish were continuously exposed to [Fluorophenyl-14C]Beta-Cyfluthrin at an average concentration of 0.12 µg eq/L for 28 days. After the exposure, the fish were transferred to flowing untreated water and the depuration of radioactivity was followed for further 28 days.
Temperature, pH and oxygen concentrations were monitored from day 0 to day 56 and were within acceptable limits; measurements ranged from 22.0 - 22.7°C, 8.0 - 8.4 and 6.1 - 8.4 mg/L, respectively.
The radioactive residues during exposure in whole fish increased rapidly (0.120 µg eq/g on day 14 and 0.121 µg eq/g on day 20) and ranged between 0.158 µg eq/g and 0.186 µg eq/g for time intervals 24 to 28 days (plateau phase). However, depuration was delayed with a depuration half-life of 8.66 days (0.052 µg eq/g on depuration day 4 and 0.017 µg eq/g on depuration day 28).

BCFss and kinetic BCFk were calculated to be 1292 and 1508, respectively. The lipid normalised BCFkL based on BCFk was 1676. The growth corrected BCF values BCFkg and BCFkLg were 1640 and 1822, respectively. All these data were based on total radioactive residues.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	[fluorophenyl-UL-14C]Beta-Cyfluthrin
Lot/Batch #:	KML 9609
Specific activity:	4.36 MBq/mg
Radiochemical purity:	> 98% (sum of isomers)
2. Vehicle and/or positive control:	Acetone
3. Test organism:	
Species:	Bluegill sunfish (*Lepomis macrochirus*)
Age:	Adult
Size: mean length:	3.75 cm
Body weight of the animals:	mean weight: 0.804 g
Loading:	initial loading of 0.32 g bodyweight/L (Based on a daily flow through volume of 250 L)
Source:	Osage Catfisheries, Inc., Osage Beach Mo 65065, USA
Diet/Food:	During the test, the fish was fed once daily (TetraMin, Tetra GmbH, D49304 Melle, containing 8.0% lipid and 48.0% total protein), based on about 2% of the average fish body weight, taking into account increasing body weights and decreasing number of fish per tank.
Acclimation period:	5 weeks in tap water

4. Environmental conditions:

Temperature:	
Control (min-max):	22.2-22.5
Treatment (min-max):	22.0-22.7
Photoperiod:	16 hours light, 8 hours dark, light intensity at light period approximately 300-400 Lux

pH:
Control (min-max): 8.1-8.4
Treatment (min-max): 8.0-8.4
Dissolved oxygen [mg/L]:
Control (min-max): 6.6-8.4
Treatment (min-max): 6.1-8.3
Total hardness:
Control: 9.5°d
Treatment: 9.0°d

B. STUDY DESIGN

1. Experimental conditions

The fish were continuously exposed to [Fluorophenol-14C] beta-cyfluthrin at an average concentration of 0.12 µg eq/L for 28 days (in µg parent equivalents/L). Due to the low water solubility of the test item and the toxicity to fish, no higher concentration could be tested. After the exposure, the fish were transferred to flowing untreated water and the depuration of radioactivity was followed for further 28 days. Temperature, pH and oxygen concentrations were monitored from day 0 to day 56 and were within acceptable limits; measurements ranged from 22.0 - 22.7 °C, 8.0 - 8.4 and 6.1 - 8.4 mg/L, respectively.

The minimal duration of the uptake phase can be calculated according to the OECD Guideline 305. Based on the log POW, the expected depuration rate constant (k_2) and the optimal duration of the uptake phase (u = 95% of steady state) are defined as:

$\log k2 = -0.414 \log (P_{OW}) + 1.47$ and $u = 3.0/k2$

Based on a log K_{OW} value of 5.9, a theoretical k_2 of 0.107 can be calculated. Based on k_2 approximately 28 days would be needed to reach 95% of "steady state". Therefore, an accumulation period of 28 days was selected which seems to be sufficient to reach steady state (= plateau level). To reach 95% depuration, theoretically a depuration period of 28 days would be needed (ln 0.01/-k_2). Therefore, a depuration period of 28 days was selected.

2. Observations

Water samples were taken from the central area of the respective test tank before feeding and immediately before fish sampling. Additionally, at selected time intervals water samples were also drawn from the corresponding mixture chamber.

During the accumulation phase, fish were sampled on Day 4, 8, 14, 20, 24 and 28. During the depuration phase, fish were sampled on day 32, 40, 48 and 56.

On each sampling occasion, six fish were collected randomly from each exposure tank, rinsed with water, sacrificed in 1.5% (v/v) 2-phenoxy-ethanol in purified water and blotted dry and weighed. At two time intervals (after 20 and 28 days of uptake) 8 fish were sampled and stored at approximately -20°C for additional analyses.

3. Calculations

Details on the calculations on depuration kinetics and bioconcentration kinetics are given in the report.

II. RESULTS AND DISCUSSION

A. FINDINGS

All validity criteria according to OECD 305 were fulfilled by meeting the following criteria:
Temperature variation was less than ± 2°C (22.0-22.7°C).
The concentration of dissolved oxygen should not fall below 60% saturation, i.e. not below 5.03 - 5.50 mg O2/L at 20 - 25°C (>6.1 mg/L).
The variation of the tank concentration during exposure was slightly higher than ± 20%. However, on one day the measured concentration was above this range (for details see below).

Additionally, the following parameters were within the required limits:
The pH values were within an acceptable range in the two tanks (8.0-8.4)
The particle content of the tap water (dry matter not passing a 0.45 µm filter) was on average 1.6 mg/L
(n=2).

The TOC value of the untreated water was on average <0.1 mg/L. A TOC value of approx. 49 mg/L can be expected from the used solvent (acetone, 0.01%). During the test (uptake day 0 to 28), the TOC value did not exceed the concentration of organic carbon originating from the test item and the solubilising agent by more than 10 mg/L (± 20%). The values were even lower than the expected value probably due to evaporation from the tanks.

The radioactive residues during exposure in whole fish increased rapidly (0.120 µg eq/g on day 14) and ranged between 0.158 µg eq/g and 0.186 µg eq/g for time intervals 24 to 28 days). However, depuration was first quick (0.052 µg eq/g on depuration day 4) and later delayed (0.017 µg eq/g on depuration day 28). The results based on total radioactive residue (TRR) are summarised as follows:

Parameter	Description	Value
kg	growth rate constant [day-1], based on depuration data	0.007
k1	overall uptake rate constant [L kg-1 day-1]	131.2
k2	overall depuration rate constant [day-1], based on depuration data	0.087
k2g	growth-corrected depuration rate constant [day-1]	0.08
CfSS	chemical concentration in fish at steady state [µg L-1]	0.155
Cw	chemical concentration in the water [mg L-1]	0.12
Ln	lipid normalisation factor	1.111
BCFSS	steady-state BCF [L kg-1]	1292
BCFSSL	lipid-normalised steady-state BCF [L kg-1]	1436
BCFk	kinetic BCF [L kg-1]	1508
BCFkL	lipid-normalised kinetic BCF [L kg-1]	1676
BCFkg	growth-corrected kinetic BCF [L kg-1]	1640
BCFkLg	lipid-normalised growth-corrected kinetic BCF [L kg-1]	1822
t0.5g	growth-corrected half-life [day]	8.66

Exposure concentration
During the accumulation period, total radioactivity levels remained sufficiently constant to show equilibrium. Total radioactivity level amounted on average to 0.12 ± 0.02 µg eq/L over the 28 days. One measurement was too high due to a malfunction in the flow system (day 22). The variation was

slightly higher than ± 20%. However, due to the need of performing a study at the very low level of 0.12 µg/L (due to low solubility and high toxicity), a slightly higher variation than required can be accepted.

Very small amounts of radioactivity were measured in the tank water during depuration. Values ranged from 0.011 µg eq/L (day 4 of depuration) to <LOQ. The measured radioactivity on day 4 of depuration reflects the on-going depuration of the radioactivity from the fish.
Radioactivity in the control tank was <LOQ at each time intervals.

Residue in fish
The radioactive residues during exposure in whole fish increased rapidly (0.120 µg/g on day 14 and 20) and ranged between 0.158 µg eq/g and 0.186 µg eq/g for time intervals 24 to 28 days. However, depuration was first quick as only 0.052 µg eq/g were measured on depuration day 4 and later delayed 0.017 µg eq/g on depuration day 28.
Taking into account the specific radioactivity of the application solution (4.36 MBq/mg), all control values for fish were < LOQ.

B. OBSERVATIONS

Two fish out of 86 fish died in the treated tank. No mortality was observed in the control tank and no symptoms were observed throughout the study.
Growth rates for the control tank and the test tank were similar with 0.012 and 0.015, respectively. The growth rate of the exposed fish differed significantly between exposure phase and depurations phase. Therefore, for the growth correction the growth rate of 0.007 of the depuration phase was used as recommended by OECD 305.

Based on the depuration data, a depuration half-life of 1.99 days was calculated. Additionally the depuration half-life, based on the k2a obtained from the uptake phase was calculated. This value was calculated to be 7.97 days. The growth corrected half-life was 8.66 days.

Mean lipid concentrations (determined in nine fish) were 41 mg/g wet weight (day 4), 45 mg/g (day 28) and 58 mg/g (day 56).
Based on the radioactivity levels in fish after exposure to [Fluorophenyl-14C]Beta-Cyfluthrin at an average dose level of 0.12 µg eq/L, the bioconcentration factor at plateau level (BCFss) and normalized to a 5% lipid content amounted to 1436.
BCFk based on Cfss, fit obtained by fitting of the uptake data resulted in a BCF value of 1508. BCFkL based on BCFk and on the lipid content measured in representative fish and normalized to a 5% lipid content amounted to 1676. The growth corrected BCF values BCFkg and BCFkLg were 1640 and 1822, respectively.
The total residue in fish consisted mainly of the parent compound (67.4% TRR on day 20 and 72.5% of the TRR on day 28). In the exposure water, the test item was measured in amounts ranging from 50.7 to 64.9% of the total radioactivity. Besides the test item, FPB acid and 4-fluoro 3-(-4-hydroxyphenoxy) benzoic acid were detected in the tank water.

III. CONCLUSION

The bioconcentration potential of beta-Cyfluthrin was investigated in Bluegill sunfish at an average exposure concentration of 0.12 µg eq/L. The plateau level, determined as the average concentration in the fish of the last two time intervals of the uptake phase, was 0.155 µg eq/g.
Because of malfunction in the flow system no reliable steady state in uptake phase was reached

(BCFss is not useful).
The lipid and growth-normalised kinetic BCF [L kg^{-1}] BCF_{kLg} is 1822.

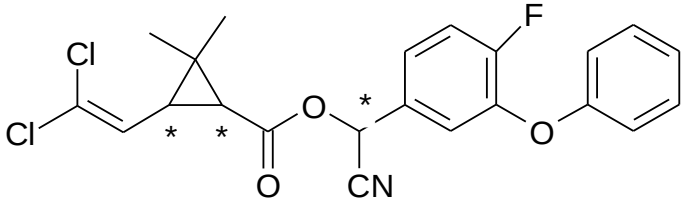
5.3.2 Summary and discussion of aquatic bioaccumulation

The Log K_{ow} values of 5.8 and 5.9 for isomer II and IV contained in beta-cyfluthrin are above the critical value of 4. Hence, according to CLP criteria beta-cyfluthrin has to be considered as potentially bioaccumulative. The experimentally derived kinetic BCF of 1822 [L kg^{-1}] for beta-cyfluthrin related to total radioactive residues and whole fish is above the trigger of 500 (criterion for bioaccumulation potential conform Regulation EC 1272/2008) f. The experimental data indicates that beta-cyfluthrin has a high potential for bioaccumulation.

5.4 Aquatic toxicity

For the evaluation of beta-cyfluthrin studies with the active substance beta-cyfluthrin as well as studies with cyfluthrin were evaluated.

The common molecular structure of cyfluthrin and beta-cyfluthrin shows three asymmetric carbon atoms (chiral centres), which leads to four diastereoisomers each consisting of an enantiomer pair.



Thus, cyfluthrin and beta-cyfluthrin are mixtures of eight isomers.
Four of the isomers are considered active: diastereoisomer II (1R,3R,1S + 1S,3S,1R = 1:1; cis) and diastereoisomer IV (1R,3S,1S + 1S,3R,1R = 1:1; trans).

The proportion of diastereoisomer pairs in cyfluthrin and beta-cyfluthrin is shown in the table below.

Table 80: Proportion of diastereoisomer pairs in cyfluthrin and beta-cyfluthrin

Diastereomer	**Cyfluthrin**	**Beta-Cyfluthrin**
I (1R-3R-R+1S-3S-S = 1:1;cis) CAS: 86560-92-1	23-27 %	< 2 %
II **(1R-3R-S + 1S-3S-R = 1:1, cis)** **CAS: 86560-93-2**	**17 -21 % (mean 19 %)**	**30-40 % (mean 35 %)**
III (1R-3R-R + 1S-3R-S = 1:1;trans) CAS: 86560-93-2	32-36 %	< 3%
IV **(1R-3S-S + 1S-3R-R = 1:1; trans)** **CAS:** **CAS: 86560-95-4**	**21-25 % (mean 22%)**	**57-67 % (mean 62 %)**
Sum of active diastereoisomers	~ 41 %	~ 97 %
Relation of II/IV	0,86	0,56

Active diastereoisomers are written in **bold**.

Therefore, cyfluthrin is 42% active isomers when compared to beta-cyfluthrin (41/97 = 42).

Therefore, the assumption is that the toxicity endpoints for beta-cyfluthrin should be 42% of the cyfluthrin endpoints.
However, the relation of the two diastereoisomers II/IV is different for cyfluthrin and beta-cyfluthrin:
II/IV (cyfluthrin) = 0.86
II/IV (beta-cyfluthrin) = 0.56
The relative activity/toxicity of diastereoisomers II and IV is unknown so far.
Generally it can be expected that beta-cyfluthrin is at least equally toxic as cyfluthrin, possibly up to 2.4 times more toxic than cyfluthrin (based on the content of biological active isomers) to aquatic organisms.

Table 81: Summary of relevant information on aquatic toxicity

Group, species **Method** **Test substance**	**Time-scale** **(Test type)**	**Endpoint**	**Toxicity** **(µg a.s./L)**	**Reference**
Fish				
Oncorhynchus mykiss OECD Guideline No. 203 Beta-cyfluthrin	96 h (flow-through) recovery 56%	Mortality, LC_{50}	0.089 (mm) [0.071 -0.107 µg/L]	Anonymous-F1,1988a (Rep. No. FF-207)
Oncorhynchus mykiss OECD Guideline No. 203 Beta-cyfluthrin	96 h (flow-through)	Mortality, LC_{50}	0.068 (mm) [0.060-0.079 µg/L]	Anonymous-F2, 1994a (Rep. No 103231)
Lepomis macrochirus OECD Guideline No. 203 Beta-cyfluthrin	96 h (flow-through)	Mortality, LC_{50}	0.280 (mm) [0.24 -0.32 µg/L]	Anonymous-F3, 1994b (Rep. No 103232)
Leuciscus idus melanotus OECD Guideline No. 203 Beta-cyfluthrin	96 h (flow-through)	Mortality, LC_{50}	0.331 (mm) [0.28 -0.399 µg/L]	Anonymous-F4, 1988b (Rep. No. FO-1011)
Oncorhynchus mykiss equivalent to EPA-FIFRA §72-4 and OECD 210 Cyfluthrin	58 d ELS (flow-through)	Growth, NOEC	0.010 (mm)	Anonymous-F5, 1985 (Rep. No. 683)
Pimephales promelas EPA-FIFRA §72-4 Cyfluthrin	307 d FLC (flow-through)	Survival parental, F1, NOEC	0.140 (mm)	Anonymous-F6, 1990 (Rep. No. 100097)
Aquatic invertebrates				
Daphnia magna OECD Guideline No. 202 Beta-cyfluthrin	48 h (semi-static)	Immobility, EC_{50}	0.105 (mm) [0.077 – 0.14 µg/L]	Kimmel, 2014a (Rep. No. D58707)
Americamysis bahia EPA-FIFRA §72-3 Beta-cyfluthrin	96 h (flow-through)	Mortality, LC_{50}	0.0022 (mm) [0.0019 – 0.0027 µg/L]	Machado,1994 (Rep. No. 106797)
Hyalella azteca EPA-FIFRA §72-3 Cyfluthrin	96 h (flow-through)	Mortality, LC_{50}	0.00055 (mm) [0.00047 – 0.00064 µg/L]	Bradley, 2013 (Rep. No. 13656.6168)
Daphnia magna OECD Guideline No. 211 Beta-cyfluthrin	21 d (semi static)	Offspring production, parental body length, NOEC	0.025 (mm)	Kimmel, 2014b (Rep. No. D58718)
Americamysis bahia OPPTS 850.1350 (EPA) Beta-cyfluthrin	28 d (flow-through)	Offspring production, parental body length, NOEC	0.00041 (mm)	Schwader, 2013 (Rep.No. 13798.6307)

Algae /aquatic plants				
Scenedesmus subspicatus OECD Guideline No. 201 Beta-cyfluthrin	96 h (static)	Biomass, E_bC_{50} Growth rate E_rC_{50} $NOE_{b/r}C$	> 10 (nom) > 10 (nom) 10 (nom)	Heimbach, 1987 (Rep.No HBF/AL 40)
Other aquatic organisms				
Chironomus riparius OECD Guideline No. 219 Beta-cyfluthrin	28 d (static, spiked water)	Emergence, NOEC	0.4 (nom)	Kimmel, 2014c (Rep. No. D58720)

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Study 1

Author:	Anonymous-F1
Title:	The acute toxicity of FCR 4545 technical to rainbow trout (*Oncorhynchus mykiss*) in a flow-through test
Date:	20 June 1988
Report no.:	FF-207
Guidelines:	EEC 79/831 Method V C.I, OECD Guideline No. 203 and EPA Pesticide Assessment Guidelines, Subdivision E, § 72-1.
GLP:	yes
Validity:	valid

Deviations: The study is valid according to the current OECD Guideline No. 203
Material: beta-Cyfluthrin techn. (FCR 4545), purity: 98.1%, batch no. 16001/87
Results: The acute toxicity of beta-cyfluthrin (FCR 4545 tech.) to rainbow trout (*Oncorhynchus mykiss*) was assessed in a flow-through test. Ten fish per test concentration were exposed for 96 hours to the following nominal concentrations: 0.063, 0.112, 0.2, 0.356 and 0.633 µg/L, along with a solvent control (acetone, 100 µL/L) and water control. The mean measured concentrations during the test were 0.053, 0.078, 0.117, 0.206 and 0.318 µg/L. The water temperature during the test was 12-13 °C, the pH was 7.6-8.1 and the oxygen content was 10.8 – 11.3 mg/L.
The LC_{50} (96h) of beta-Cyfluthrin technical (FCR 4545) was 0.089 µg a.s./L with a 95% confidence interval of 0.071 – 0.107 µg a.s./L (based on mean measured concentrations). The lowest lethal concentration (LLC) was 0.078 µg a.s./L. The no observed effect concentration (NOEC) was 0.053 µg a.s./L. All values are based on mean measured concentrations. There were no mortality or sublethal effects in the control and solvent control, respectively. The validity criteria of OECD 203 are fulfilled.

Conclusion: LC_{50} (96h, flow-through) = 0.089 µg a.s./L

Study 2

Author:	Anonymous-F2
Title:	Acute toxicity of FCR 4545 technical to rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions
Date:	24 August 1994
Report no.:	103231
Guidelines:	US-EPA FIFRA § 72-1 Guideline
GLP:	yes
Validity:	valid

Deviations: The study is valid according to the current OECD Guideline No. 203
Material: beta-Cyfluthrin techn. (FCR 4545), purity: 99.4%, batch no. 88R0256I
Results: The acute toxicity of beta-cyfluthrin (FCR 4545 tech.) to rainbow trout (*Oncorhynchus mykiss*) was assessed in a flow-through test. Twenty fish per test concentration (two replicates of 10 fish each) were exposed for 96 hours to the following nominal concentrations: 0.039, 0.065, 0.11, 0.18 and 0.3 µg/L, along with a solvent control (acetone, 0.014 mL/L) and water control. The mean measured concentrations during the test were 0.039, 0.051, 0.083, 0.16 and 0.2 µg/L. The water temperature during the test was 12-13 °C, the pH was 7.2-7.3 and the oxygen content was 9.2 – 10.2 mg/L.
The 96h LC_{50} value of beta-Cyfluthrin technical was 0.068 µg a.s./L with a 95% confidence interval of 0.060 – 0.079 µg a.s./L (based on mean measured concentrations). The no observed effect concentration (NOEC) was < 0.039 µg a.s./L. All values are based on mean measured concentrations. There were no mortality or sublethal effects in the control and solvent control, respectively. The validity criteria of OECD 203 are fulfilled.

Conclusion: LC_{50} (96 h, flow-through) = 0.068 µg a.s./L

Study 3

Author:	Anonymous-F3
Title:	Acute toxicity of FCR 4545 technical to bluegill (*Lepomis macrochirus*) under flow-through conditions
Date:	24 August 1994
Report no.:	103232
Guidelines:	US-EPA FIFRA § 72-1 Guideline
GLP:	yes
Validity:	valid

Deviations: The study is valid according to the current OECD Guideline No. 203
Material: beta-Cyfluthrin techn. (FCR 4545), purity: 99.4%, batch no. 88R0256I
Results: The acute toxicity of beta-cyfluthrin (FCR 4545 tech.) to bluegill (*Lepomis macrochirus*) was assessed in a flow-through test. Twenty fish per test concentration (two replicates of 10 fish each) were exposed for 96 hours to the following nominal concentrations: 0.19, 0.32, 0.54, 0.9 and 1.5 µg/L, along with a solvent control (acetone, 0.014 mL/L) and water control. The mean measured concentrations during the test were 0.11, 0.22, 0.31, 0.55 and 0.79 µg/L. The water temperature during the test was 22-23 °C, the pH was 7.1-7.2 and the oxygen content was 7.9 – 8.4 mg/L.
The 96h LC_{50} value of beta-Cyfluthrin technical was 0.28 µg a.s./L with a 95% confidence interval of 0.24 – 0.32 µg a.s./L (based on mean measured concentrations). The no observed effect

concentration (NOEC) was 0.11 µg a.s./L. All values are based on mean measured concentrations. There were no mortality or sublethal effects in the control and solvent control, respectively. The validity criteria of OECD 203 are fulfilled.

Conclusion: LC_{50} (96 h, flow-through) = 0.28 µg a.s./L

Study 4

Author:	Anonymous-F4
Title:	The acute toxicity of FCR 4545 technical to golden orfe (*Leuciscus idus melanotus*) in a flow-through test
Date:	31 May 1988
Report no.:	FO-1011
Guidelines:	EEC 79/831 Method V C.I, OECD Guideline No. 203 and EPA Pesticide Assessment Guidelines, Subdivision E, § 72-1
GLP:	Yes
Validity:	valid

Deviations: The study is valid according to the current OECD Guideline No. 203
Material: beta-Cyfluthrin techn. (FCR 4545), purity: 98.1%, batch no. 16001/87
Results: The acute toxicity of beta-cyfluthrin (FCR 4545 tech.) to golden orfe (*Leuciscus idus melanotus*) was assessed in a flow-through test. Ten fish per test concentration were exposed for 96 hours to the following nominal concentrations: 0.124, 0.221, 0.394, 0.7 and 1.245 µg/L, along with a solvent control (acetone, 100 µL/L) and water control. The mean measured concentrations during the test were 0.0836, 0.1988, 0.227, 0.496 and 0.689 µg/L. The water temperature during the test was 20-22 °C, the pH was 7.8-8.1 and the oxygen content was 10.3 – 11.2 mg/L.
The 96h LC_{50} of beta-Cyfluthrin technical was 0.331 µg a.s./L with a 95 % confidence interval of 0.2801 – 0.3987 µg a.s./L (based on mean measured concentrations). The lowest lethal concentration (LLC) was 0.496 µg a.s./L. The no observed effect concentration (NOEC) was 0.1988 µg a.s./L. All values are based on mean measured concentrations. There were no mortality or sublethal effects in the control and solvent control, respectively. The validity criteria of OECD 203 are fulfilled.

Conclusion: LC_{50} (96 h, flow-through) = 0.331 µg a.s./L

5.4.1.2 Long-term toxicity to fish

No reliable long-term study conducted with beta-Cyfluthrin is available. For the chronic effects to fish, reference is made to studies with Cyfluthrin.

Study 1

Author:	Anonymous-F5
Title:	Toxicity of Cyfluthrin (Baythroid) technical to early life stages of rainbow trout
Date:	24 October 1985
Report no.:	683
Guidelines:	Equivalent to the test guidelines US-EPA FIFRA § 72-4 guideline and OECD Guideline No. 210

GLP:	Yes
Validity:	Valid

Deviations: The study is valid according to the current OECD 210 guideline, with some minor deviations not influencing the outcome of the study. The dissolved oxygen concentration stayed within the targeted limits of 6.5 to 11.9 ppm (three occasions above that range). The temperature during the study ranged from 8.3 to 11.9°C (recommended 8.5 to 11.5).
Test material: Cyfluthrin techn. (Baythroid), purity: 96.0%, batch no. 84-R-221-1/7

Results: The early life stages of rainbow trout (*Oncorhynchus mykiss*) were exposed to five concentrations of Cyfluthrin for 58 days, i.e. nominal 0.025, 0.050, 0.100, 0.200 and 0.400 µg/L.
The mean measured concentration ranged from 32% to 48% of nominal.
During the period of exposure, there were no concentration-related embryonic deaths, but larvae mortality at the three highest concentrations tested. Growth as measured by biomass and mean fish weight was significantly reduced in the 0.050, 0.100, 0.200 µg/L groups (0.0177, 0.0318 and 0.0848 µg/L based on mean measured concentrations). The number of fish showing behavioural signs at concentrations of nominal 0.050 µg/L and higher was statistically significant increased.
The no observed effect concentration was 0.010 µg/L (based on mean measured concentration) or 0.025 µg/L (nominal).
Due to the significantly reduced number of swim ups (only 10 %) followed by death rate of 100 % (0.4 µg/L nominal) and still high mortalities for the next lower applied concentrations, it can be assumed that the toxic effects are mainly attributed to the exposure of fish embryos (fish eggs) within the hatching stage.

Conclusion:
The LC_{50} (58 d) of Cyfluthrin is 0.069 µg/L (mm).

The **NOEC (58 d) of Cyfluthrin is 0.01 µg/L (mm)**.

Study 2

Author:	Anonymous-F6
Title:	Full Life-Cycle Toxicity of 14C-Cyfluthrin (Baythroid®) to the Fathead Minnow (*Pimephales promelas*) Under Flow-Through Conditions
Date:	2 April 1990
Report no.:	100097
Guidelines:	US-EPA FIFRA § 72-4 guideline, 40 CFR, Section 158.145
GLP:	Yes
Validity:	Valid

Deviations: The study is valid according to the current US EPA protocol OPPTS 850.1500 Fish life cycle toxicity. A mortality rate of 37.5 % in the control group 153 -301 days post-hatch was determined. Hence, data about survival 153 -301 d considered as not fully reliable.

Test material: 14C-Cyfluthrin (Baythroid), purity: 99.0%, reference no. PS-2344

Results: The study was initiated using newly fertilized eggs (<24 hours post-fertilization) with

exposure continuing for 301 days post-hatch. Mean measured exposure concentrations, determined by liquid scintillation counting techniques (LSC), were 0.018, 0.033, 0.065, 0.14 and 0.29 µg a.s./L. These mean values ranged from 106 to 116% of the nominal concentrations of 0.016, 0.031, 0.063, 0.13 and 0.25 µg a.s./L. Of the 82% average 14C-activity recovered, 90% was characterised as ^{14}C-Cyfluthrin.
No significant difference in parental generation hatchability was exhibited, while F1 egg hatchability was significantly reduced at the highest concentration tested. Survival, in both parental and F1 generations, was significantly reduced at approximately 60-days post-hatch at the highest concentration tested. Survival was not significantly reduced at any other interval. Growth, as reflected by standard length and wet weight was not significantly reduced compared to the control in either the parental or F1 generation at any concentration tested. Reproductive success, as measured by the number of spawns, number of eggs, number of eggs/spawn, number of reproductive days and the number of eggs/pair/reproductive day, was not significantly reduced at any of the four concentrations examined.
The no observed effect concentration (NOEC) was 0.14 µg a.s./L (based on mean measured concentration or nominal 0.13 µg a.s./L).

Conclusion: The **NOEC (mm) of Cyfluthrin is 0.14 µg/L**.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Study 1

Author:	Kimmel, S.
Title:	Beta-Cyfluthrin: Acute toxicity to *Daphnia magna* in a 48- Hour Immobilization Test
Date:	19 March 2014
Report no.:	D58707
Guidelines:	OECD Guideline No. 202
GLP:	Yes
Validity:	Yes

Deviations: None
Dates of experimental work: 25 September 2012 to 07 March 2013

Executive Summary
The acute toxicity of beta-Cyfluthrin to *Daphnia magna* was determined in a 48-hour semi-static test. Twenty *Daphnia* (4 replicates of 5 animals per test beaker) per concentration were exposed to 0.01, 0.032, 0.1, 0.32 and 1.0 µg a.s./L nominal concentrations. In addition, 4 x 5 *Daphnia* were exposed to a test water control (without test item) and a solvent control (60 µL DMF/L).
Daphnids were observed for immobilisation at 24 and 48 hours and were not fed during the test. The measured concentrations of beta-Cyfluthrin in the freshly prepared test media were between 40 and 75% of nominal. The measured concentrations in the aged test medium at the end of the renewal periods were between 37 and 71% of nominal. Therefore, all results are based on mean measured concentrations.
All validity criteria according to the guideline OECD 202 were fulfilled. However, as the effects at

0.05 µg/L (mm) and 0.16 µg/L (mm) increase from 0 % to 85 % the chosen spacing of test concentrations is regarded to be less appropriate.

Conclusion:
The 48-h EC_{50} for *Daphnia magna* exposed to beta-Cyfluthrin based on mean measured concentration was 0.105 µg/L with a 95% confidence interval of 0.077 to 0.14 µg/L. No effect on immobilisation was reported up to 0.05 µg/L.

Study 2

Author:	Machado, M.W.
Title:	Acute toxicity of FCR 4545 to the Mysid Shrimp (*Mysidopsis bahia*) Under Flow Through Conditions
Date:	17 October 1994
Report no.:	106797
Guidelines:	US-EPA FIFRA § 72-3 guideline
GLP:	Yes
Validity:	Valid

Deviations: None

Executive Summary
The effects of beta-Cyfluthrin (FCR 4545, 14C-labelled) on *Mysidopsis bahia* were evaluated in a 96-hour flow-through toxicity test. Twenty shrimps (2 replicates of 10 animals per test beaker) per concentration were exposed to 0.65, 1.1, 1.8, 3.0 and 5.0 ng/L beta-Cyfluthrin nominal concentrations. In addition, 2 × 10 mysid shrimps were exposed to test water without test substance (blank control) and to a solvent control. Shrimps were observed for mortality and sublethal effects at test initiation and after 24, 48, 72 and 96 hours.
The analysed test concentrations ranged between 72% and 96% of the nominal concentrations (mean of replicates and 0-hour and 96-hour analysis). Therefore, the results reported are related to mean measured test concentrations, i.e. 0.61, 0.96, 1.3, 2.3 and 3.8 ng/L beta-Cyfluthrin.
All validity criteria according to US EPA FIFRA Guideline 72-3 were fulfilled.

Conclusion:
The 96-h LC50 for *Americamysis bahia* exposed to beta-Cyfluthrin based on mean measured concentration was 2.2 ng/L with a 95% confidence interval of 1.9 to 2.7 ng/L.

I. MATERIALS AND METHODS
A. MATERIALS
1. Test material:

Test item:	[penyl-U-14C]beta-Cyfluthrin (FCR 4545)
Lot/Batch #:	C-652A
Radiopurity:	> 98%
Specific Activity:	56.7 mCi/mmol

2. Vehicle and/or positive control:

Test water:	Seawater collected from Cape Cod Canal, Bourne, Massachusetts, USA
Solvent:	Acetone

3. Test organisms:

Species:	Americamysis bahia
Source:	Laboratory bred (Lot# 94A45a), purchased from commercial supplier, FT. Collins, Colorado
Loading:	10 organisms per vessel

4. Environmental conditions:

Temperature:	21 to 22°C
Photoperiod:	Light/dark 16/8 hours
Light intensity:	290 to 970 lux
pH:	Start of the test: 8.0
	End of the test: 7.8 - 7.9
Dissolved oxygen:	Start of the test: 6.1 – 6.9 mg O2/L
End of the test:	5.9 – 6.7 mg O2/L
Salinity:	32‰

B. STUDY DESIGN

1. Experimental treatments

The effects of beta-Cyfluthrin on *Mysidopsis bahia* were evaluated in a 96-hour flow-through toxicity test using 14C-labelled test item. Twenty shrimps (2 replicates of 10 animals per test beaker) per concentration were exposed to 0.65, 1.1, 1.8, 3.0 and 5.0 ng/L nominal concentrations. In addition, 2 × 10 mysid shrimps were exposed to test water without test substance (blank control) and to a solvent control.

2. Observations

Shrimps were observed for mortality and sublethal effects at test initiation and after 24, 48, 72 and 96 hours. Live brine shrimp nauplii (*Artemia salina*) were added to each test vessel containing live test organisms twice daily *ad libitum*. Dissolved oxygen concentrations, pH, salinity and temperature were measured once daily in both replicates of each treatment level and the controls. In addition, the temperature was continuously monitored in one replicate. Samples for the determination of beta-Cyfluthrin in the test medium were taken from both replicates of the blank control and the test concentrations before test start and at test start and after 96 hours from each replicate of each treatment level.

All samples were extracted and analysed for [14C]FCR 4545 using a liquid scintillation counting (LSC) procedure according. In addition to the exposure solution analyses, thin layer chromatography (TLC) was used to determine the radiopurity of the high test concentration (5.0 ng/L), the primary radiolabelled stock solution and the diluter stock solution.

3. Statistical calculations

The 96-hour LC50 value was determined by probit analysis including 95% confidence limits.

II. RESULTS AND DISCUSSION

A. FINDINGS

Based on mean measured concentrations of [14C]beta-Cyfluthrin, the following LC50 values for mortality after 96 hours of flow-through exposure were assessed.

Table 82: Results for mortality of mysids for [14C]beta-Cyfluthrin

[14C]beta-Cyfluthrin			
Timepoint	**value [ng a.s./L, mean measured]**	**lower 95% cl [µg a.s./L, mean measured]**	**upper 95% cl [µg a.s./L, mean measured]**
96-hour LC_{50}	2.2	1.9	2.7
96-hour NOEC	1.3	-	-

Analytical data: The analysed test concentrations of [14C]beta-Cyfluthrin ranged between 72 – 96% of nominal treatment levels. The mean measured test concentrations were 0.61, 0.96, 1.3, 2.3 and 3.8 ng/L.

B. OBSERVATIONS

Mortality started at 1.3 ng /L beta-Cyfluthrin (mean measured concentrations). In addition, sublethal effects (i.e., loss of equilibrium, lethargy) were observed among surviving mysids exposed to the 2.3 and 3.8 ng/L treatment levels.

Table 83: Toxicity of [14C]beta-Cyfluthrin to *Americamysis bahia*

Nominal test concentration [ng a.s./L]	Mean measured concentration [ng a.s./L]	Number of exposed mysid shrimp per replicate	Cumulative mean Mortality [%]			
			24-hours	48-hours	72-hours	96-hours
Control	-	20	0	0	0	0
Solvent control	-	20	0	0	0	0
0.64	0.61	20	0	0	0	0
1.1	0.96	20	0	0	0	0
1.8	1.3	20	0	0	0	0
3.0	2.3	20	10 ^{BCD}	25 ^{BFGH}	45 ^{BK}	55 ^{BEL}
5.0	3.8	20	20 ^{AB}	65 ^{BE}	85 ^{DIJ}	90 ^{DJ}

All validity criteria according to the FIFRA Guideline 72-3 were fulfilled, as less than 10% mortality of mysid shrimps was observed in control groups.

III. CONCLUSION

The 96-h LC50 for *Americamysis bahia* exposed to [14C] beta-Cyfluthrin based on mean measured concentration was 2.2 ng/L with a 95% confidence interval of 1.9 to 2.7 ng/L.

Study 3

Author:	Bradley, M., J.
Title:	Cyfluthrin - Acute Toxicity to Freshwater Amphipods (*Hyalella azteca*) Under Flow-Through Conditions
Date:	24 June 2013
Report no.:	13656.6168
Guidelines:	US-EPA FIFRA § 72-3 guideline
GLP:	Yes
Validity:	Valid

Deviations: None

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item: Cyfluthrin technical
Lot/Batch #: FHER904218
Radiopurity: 95.8%

2. Vehicle and/or positive control:

Test water: laboratory well water

Solvent: Acetone

3. Test organisms:

Species: *Hyalella azteca* SMV Lot No. 011713, eight days old at test initiation

Source:	Smithers Viscient culture
Loading:	10 organisms per vessel

4. Environmental conditions:

Temperature:	22 - 24 °C
Photoperiod:	Light/dark 16/8 hours
Light intensity:	100 to 500 lux
pH:	Start of the test: 7.2
	End of the test: 7.2 – 7.4
Dissolved oxygen:	Start of the test: 8.7 – 9.2 mg O_2/L
End of the test:	7.5 – 9.5 mg O_2/L

B. STUDY DESIGN

1. Experimental treatments

The effects of Cyfluthrin on *Hyalella azteca* were evaluated in a 96-hour flow-through toxicity test. Twenty amphipods (2 replicates of 10 animals per test beaker) per concentration were exposed to 0.20, 0.40, 0.80, 1.6 and 3.2 ng a.i./L nominal concentrations. In addition, 2 × 10 amphipods were exposed to test water without test substance (blank control) and to a solvent control.

2. Observations

The number of dead *Hyalella* in each test vessel was recorded at test initiation and after 24, 48, 72 and 96 hours of exposure. Death was determined by gently agitating the test solution around those amphipods that appeared to be immobile. If no physical response was observed, immobile *Hyalella* were inspected within a pipette and closely examined for any subtle physical movements (e.g. slight movements of the appendages). If upon further inspection there was no observed movement, the *Hyalella* were considered dead. Additional cues in combination with immobility, such as discoloration and decay, were also used to determine mortality. Biological observations and observations of the physical characteristics of each replicate test solution were made and recorded at test initiation and after 24, 48, 72 and 96 hours of exposure.

Prior to the start of the definitive exposure, samples were removed from all treatment level, control and solvent control solutions and analysed for Cyfluthrin concentrations. Results of the pretest analyses were used to judge whether sufficient quantities of Cyfluthrin were being delivered to the test vessels and whether the appropriate test concentrations were being maintained in order to initiate the definitive exposure.

During the in-life phase of the definitive study, one sample was removed from each test, control and solvent control solution for analysis of Cyfluthrin concentration at 0 hour (test initiation) and 96 hours (test termination). Samples were collected from the approximate midpoint of the test vessels by siphoning. Samples analysed at test initiation (0 hour) and test termination (96 hour) were a composite of both replicates of each test level, control and solvent control.

Exposure solutions and QC samples were analysed for Cyfluthrin using gas chromatography with mass selective detection (GC/MSD).

3. Statistical calculations

A computer program, CETIS-Comprehensive Environmental Toxicity Information System™ (Ives, M., 2011), will be used to estimate LC50 values. An LC50 value cannot be calculated if the mortality data derived is insufficient. The method selected is determined by the data base (i.e., presence or absence of 100% response, number of partial responses, etc.).

II. RESULTS AND DISCUSSION

A. FINDINGS

Based on mean measured concentrations of Cyfluthrin technical, LC50 values for mortality after 96 hours of flow-through exposure were assessed.

Table 84: Results for mortality of *Hyalella azteca* for cyfluthrin

Cyfluthrin			
Timepoint	**value [ng a.s./L, mean measured]**	**lower 95% cl [ng a.s./L, mean measured]**	**upper 95% cl [ng a.s./L, mean measured]**
96-hour LC_{50}	0.55	0.47	0.64

Analytical data: The analysed test concentrations of Cyfluthrin ranged between 76 – 87 % of nominal treatment levels. The mean measured test concentrations were 0.17, 0.32, 0.66, 1.2 and 2.4 ng/L.

B. OBSERVATIONS

Mean measured concentrations, percent mortality, and observations recorded during the 96-hour definitive test are presented in Table 64. Following 96 hours of exposure, 10, 10, 70, 100 and 100% mortality was observed among *Hyalella azteca* exposed to mean measured concentrations of 0.17, 0.32, 0.66, 1.2 and 2.6 ng/L, respectively. All surviving *Hyalella* exposed to the 0.66 ng/L treatment level were observed to be lethargic. Following 96 hours of exposure, 5% mortality was observed among *Hyalella* exposed to the control while no mortality or adverse effects were observed among *Hyalella* exposed to the solvent control.

Table 85: Toxicity of Cyfluthrin to *Hyalella azteca*

Nominal test concentration [ng a.s./L]	**Mean measured concentration [ng a.s./L]**	**Number of exposed mysid shrimp per replicate**	**Cumulative mean Mortality [%]**			
			24-hours	**48-hours**	**72-hours**	**96-hours**
Control	-	20	0	0	5	5
Solvent control	-	20	0	0	0	0
0.20	0.17	20	0	5[a]	5	10
0.40	0.32	20	5	5	5	10
0.80	0.66	20	20[b]	45[ac]	65[ac]	70[b]
1.6	1.2	20	35[d]	50[d]	95[e]	100
3.2	2.4	20	50[d]	65[e]	95[e]	100

a Several *H. azteca* were observed to be lethargic.
b All surviving *H. azteca* were observed to be lethargic.
c Two *H. azteca* were observed to be immobilized.
d Several *H. azteca* were observed to be immobilized.
e All surviving *H. azteca* were observed to be immobilized.

III. CONCLUSION

The 96-h LC50 for *Hyalella azteca* exposed to Cyfluthrin based on mean measured concentration was 0.55 ng/L with a 95% confidence interval of 0.47 to 0.64 ng/L.
This study is considered as the most relevant for acute toxicity to aquatic invertebrates, because considering the acute effect data for cyfluthrin, the standard species *Daphnia magna* has been two magnitudes less sensitive than the most sensitive non-standard species *Hyalella azteca*. Unfortunately there is no study with *Hyalella azteca* for beta-cyfluthrin available. But generally it can be expected that beta-cyfluthrin is at least equally toxic as cyfluthrin, possibly up to 2.4 times more toxic than

cyfluthrin (based on the content of biological active isomers). Therefore the study with cyfluthrin is considered as relevant for the assessment of beta-cyfluthrin and the results support those of the acute study with *Americamysis bahia*.

5.4.2.2 Long-term toxicity to aquatic invertebrates

Study 1

Author:	Kimmel, S.
Title:	Influence of Beta-Cyfluthrin (techn.) on the reproduction rate of water fleas (*Daphnia magna*)
Date:	19 March 2014b
Report no.:	D58718
Guidelines:	OECD Guideline No. 211, *Daphnia magna* Reproduction Test, October 03, 2008, EU Commission Directive 92/69/EEC, part C.20, *Daphnia magna* Reproduction Test (2001), EU Commission Regulation (EC) No 440/2008, C.20: "*Daphnia magn*a Reproduction Test.
GLP:	yes
Validity:	valid

Deviations: None
Dates of experimental work: 27 November 2012 to 14 March 2013
Executive Summary
The effect of the test item beta-Cyfluthrin on the survival and reproduction of *Daphnia magna* was investigated in a semi-static test over 21 days. Ten Daphnia (1 animals per test beaker) per concentration were exposed to 0.001, 0.0032, 0.010, 0.032 and 0.10 µg a.s./L nominal concentrations. In addition, 10 Daphnia were exposed to a test water control (without test item) and a solvent control (60 µL DMF/L).
Daphnids were observed for immobilization and reproduction on Days 0-2, 5, 7, 9, 12, 14, 16, 19 and 21 and were fed daily during the test. Samples for the determination of the concentrations of beta-Cyfluthrin in the test medium were taken from the test concentrations 0.010, 0.032 and 0.10 µg a.s./L and from the solvent control of the first, second and last week of the test, i.e. on day 0, 7 and 16, respectively. Samples for the determination of the stability of beta-Cyfluthrin were taken at the end of two test medium renewal periods of 48 hours (days 2 and 9) and at the end of one renewal period of 72 hours (day 19).
In the application solutions of each measured renewal period, the test item concentrations were between 96% and 107% of the nominal values throughout the sample period. Thus, the test item was stable in the application solutions throughout each of the renewal periods. The measured concentrations in the freshly prepared test media of the nominal concentrations of 0.010, 0.032 and 0.10 µg a.s./L were between 40 and 133% of nominal values at the start of the test medium renewal periods. In the stability control samples without food particles and daphnids, the measured concentrations were between 23 and 109% of the nominal values at the end of the test medium renewal periods of 48 to 72 hours. All validity criteria according to the guideline OECD 211 were fulfilled.

Conclusion:
The highest mean measured concentration of beta-Cyfluthrin tested without effects after the exposure period of 21 days (21-day NOEC) based on reproduction was 0.025 µg a.s./L.

The lowest concentration tested with effects (21-day LOEC) was determined to be 0.057 µg a.s./L (mean measured). The 21-d EC_{10}, EC_{20} and EC_{50} for *Daphnia magna* exposed to beta-Cyfluthrin based on mean measured concentrations were 0.023, 0.041 and >0.057 µg/L, respectively and with 95% confidence intervals of 0.0017 to 0.034 µg/L, 0.020 to >0.057 µg/L and 0.076 to >0.057 µg/L, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Beta-Cyfluthrin
Lot/Batch #:	PNBC000623
Purity:	99.3% w/w

2. Vehicle and/or positive control: N,N-Dimethylformamide (DMF)

3. Test organisms:

Species:	Daphnia magna
Age:	First instars (< 24 h old)
Source:	Laboratory bred
Loading:	1 organisms per vessel (100 mL glass beakers containing 80 mL test solution)

4. Environmental conditions:

Temperature:	20 to 21 C°
Photoperiod:	Light/dark 16/8 h
Light intensity:	400 to 540 lux
pH:	7.5 to 8.0
Dissolved oxygen:	7.9 to 9.0 mg O2/L
Hardness:	250 mg/L CaCO3
Alkalinity:	0.9 mmol/L

B. STUDY DESIGN

1. Experimental treatments

The effects of beta-Cyfluthrin on immobilization and reproduction of *Daphnia magna* were evaluated in a 21 days semi-static toxicity test. Ten *Daphnia* (1 animals per test beaker) per concentration were exposed to 0.001, 0.0032, 0.01, 0.032 and 0.1 µg a.s./L nominal concentrations.

In addition, ten *Daphnia* were exposed to a test water control (without test item) and a solvent (60 µL DMF/L) control. The *Daphnia* were randomly placed into the test beaker and exposed to the test item for 21 days. The test media of all treatments were renewed on days 2, 5, 7, 9, 12, 14, 16 and 19 of the test period. The test animals were fed daily with a food mixture containing a suspension of green algae of the species *Scenedesmus subspicatus* and a fish food suspension.

2. Observations

The test replicates were observed for immobility of adults on days 0-2 and thereafter on day 5, 7, 9, 12, 14, 16, 19 and 21 before renewal of the test media. On the same dates, the test replicates were observed for the number of living and dead offspring and for the presence of aborted eggs. The reproduction rate was calculated as the total number of living offspring produced per parent female surviving until the end of the test pH-values and dissolved oxygen concentrations were determined in the test media at the beginning and the end of each renewal period. The water temperature was measured in one of the control replicates at the same time. Samples for the determination of the

concentrations of beta-Cyfluthrin in the test medium were taken from all test concentrations and from the solvent control of the first, second and last week of the test, i.e. on day 0, 7 and 16, respectively. Samples for the determination of the stability of beta-Cyfluthrin were taken at the end of two test medium renewal periods of 48 hours (days 2 and 9) and at the end of one renewal period of 72 hours (day 19).

3. Statistical calculations

The mean reproduction rates of the daphnids at the test concentrations were compared to the pooled controls by multiple Williams t-tests. Additionally, the EC10, EC20 and EC50 for the inhibition of the reproduction rate after 21 days were calculated by Probit Analysis.

II. RESULTS AND DISCUSSION

A. FINDINGS

Based on the mean measured concentrations of beta-Cyfluthrin, the following endpoints for reproduction of the test animals after 21 days of semi-static exposure were assessed.

Table 86: Results for inhibition of reproduction for beta-cyfluthrin

Endpoints	Inhibition of reproduction rate (21 days, mean measured concentration)
EC10 [µg a.s./L] 95% confidence interval	0.023 0.0017 – 0.034
EC20 [µg a.s./L] 95% confidence interval	0.041 0.020 - > 0.057*
EC50 [µg a.s./L] 95% confidence interval	> 0.057* 0.076 - > 0.057
NOEC [µg a.s./L]	0.025
LOEC [µg a.s./L]	0.057

* Extrapolated value, EC_{50} and upper confidence interval are greater than the highest concentration tested

Analytical data:

In the application solution samples, the measured test item concentrations ranged from 101% to 107% of nominal in the freshly prepared application solutions (measured on days -1, 5, 12 and 19), and from 96% to 103% in the aged application solutions (measured on days 2, 9 and 16). Thus, the test item was stable in the application solutions throughout each of the renewal periods. The measured concentrations in the freshly prepared test media of the nominal concentrations of 0.010, 0.032 and 0.10 µg a.s./L were between 40 and 133% of nominal values at the start of the test medium renewal periods. In the stability control samples without food particles and daphnids, the measured concentrations were between 23 and 109% of the nominal values at the end of the test medium renewal periods of 48 to 72 hours.

B. OBSERVATIONS

The survival of *Daphnia magna* after 21 days was reduced at the mean measured concentrations of 0.025 and 0.057 µg a.s./L, which was however, not statistically significant reduced compared to pooled controls. The time of the first brood was not affected by the test item up to the mean measured concentration of
0.025 µg a.s./L.

The mean reproduction rate of the daphnids in the solvent control was 120 ± 18.4 living offspring per surviving adult. The corresponding value in the control was 133 ± 6.7.

No significant effect on reproduction was determined up to and including the mean measured test concentration of 0.025 µg a.s./L (Williams t-test, one-sided smaller, $\alpha = 0.05$). At the highest concentration of 0.057 µg a.s./L (mean measured), the mean reproduction rate of surviving daphnids

was statistically significantly reduced. At this concentration the mean reproduction rate was 92±19.4 living offspring per surviving adult (73% compared to the pooled controls).
With the exception of the reported mortality and reduced reproduction rates, no visible abnormalities were observed at the test animals during the test.

Table 87: Summary of mortality and reproduction rates

	Solvent Control	**Control**	**Beta-Cyfluthrin nominal (and measured) concentration [µg a.s./L]**				
			0.0010 (n.a.)	**0.0032 (n.a.)**	**0.010 (0.0059)**	**0.032 (0.025)**	**0.10 (0.057)**
Mortality [%] after 21 days of exposure	10	0	0	0	0	30	30
Mean reproduction rate (living offspring per surviving adult)	120.3	132.9	140.7	147.7	141.5	114.7	92.3*
Mean reproduction rate in % of pooled conrols	100.0		110.8	116.5	111.4	90.3	72.7

Note: Both controls (water and solvent) were pooled for statistical analysis. Mean reproduction rates are referred to the results of the pooled controls.
* statistically significantly lower than the pooled controls value, results of a Williams t-test, one-sided smaller, $\alpha = 0.05$
n.a.: not analyzed since below NOEC of the study.

The test is considered to be valid, as in the control and solvent control the survival of the parent animals at the end of the test was 100% and 90%, respectively. Furthermore, the mean number of live offspring produced per parent animal surviving at the end of the test is > 60 in the control and solvent control.

III. CONCLUSION

The highest mean measured concentration of beta-Cyfluthrin tested without effects after the exposure period of 21 days (21-day NOEC) based on reproduction was 0.025 µg a.s./L.
The lowest concentration tested with effects (21-day LOEC) was determined to be 0.057 µg a.s./L (mean measured). The 21-d EC_{10}, EC_{20} and EC_{50} for *Daphnia magna* exposed to beta-Cyfluthrin based on mean measured concentrations were 0.023, 0.041 and >0.057 µg/L, respectively and with 95% confidence intervals of 0.0017 to 0.034 µg/L, 0.020 to >0.057 µg/L and 0.076 to >0.057 µg/L, respectively.

Study 2

Author:	Schwader, A.L.
Title:	Beta-Cyfluthrin –Life Cycle Toxicity Test with Mysids (*Americamysis bahia*)
Date:	18 September 2013
Report no.:	13798.6307
Guidelines:	OPPTS Draft Guideline 850.1350
GLP:	yes
Validity:	valid

Deviations: None
At test termination, the mysids in the control and solvent control met the performance criteria of the OPPTS 850.1350 guideline (> 70% survival of F0 mysids between pairing and exposure termination, >75% of the females in the control and solvent control released young, and the control and solvent control organisms produced > 3 offspring per female). Post-pairing survival for the control and solvent control mean was 95% and 89%, respectively. Percentage of reproductively active females for both control and solvent control mysids was 100% for all replicates. The reproduction of mysids exposed to the control and solvent control ranged from 19.0 to 24.8 and 20.2 to 27.6 offspring per female, respectively. The control and solvent control mean was 21.5 and 23.8 offspring per female, respectively. No behavioural abnormalities were observed during the exposure period.

Materials:

Test item: Beta-Cyfluthrin (BCS-AH45780)
Purity: 99.2% w/w;
Batch: ABIDBBB085;
Test organism: Mysids *(Americamysis bahia)*, ≤ 23 hours old.

Study Design and Methods:

Mysids were exposed in a chronic test for 28 days under flow-through conditions to five nominal test concentrations of 0.25, 0.50, 0.99, 2.0 and 4.0 ng/L (corresponding to mean measured concentrations of 0.11, 0.23, 0.41, 0.83 and 1.5 ng/L), a dilution water control and solvent control (acetone). Four replicates were maintained for each treatment and the controls. Each exposure aquarium contained one retention chamber, yielding 20 mysids per replicate vessel.
Survival of mysids (F0 generation) was estimated until day 12 (due the rapid movement of mysids in a single chamber containing up to 20 mysids) and counted thereafter daily. At day 12 mature mysids were paired. During the reproductive phase groups of 10 offspring F1 mysids per replicate (40 per treatment) were placed in a separate pairing chamber and monitored for 96 hours post-release for survival and behaviour. For each replicate aquarium the total number of offspring produced per female was assessed. Furthermore the mean total body length and the dry weight of the parental generation were determined. Test conditions: 28-day duration, temperature range of 26 to 28 ºC, illumination of 16 hours light (270 to 390 lux) and 8 hours darkness. Diluted, filtered, natural seawater (salinity range of 19 to 21‰ and a pH range of 7.7 to 8.2) was used as dilution and control water.
Dates of experimental work: May 03, 2013 to May 31, 2013

Results and Discussion:

Analytical results

Mean measured concentrations of beta-Cyfluthrin in the five test levels ranged from 37% to 46% of nominal concentrations. Based on mean measured beta-Cyfluthrin concentrations, the treatment levels are defined as follows: 0.11, 0.23, 0.41, 0.83 and 1.5 ng/L.

Biological results

Table 88: Summary of the first generation (F0) survival at termination of the 28-day life-cycle exposure of mysids (*Americamysis bahia*) and of F1 survival at 96-hours post release following of mysids (*Americamysis bahia*) to beta-Cyfluthrin

Mean Measured concentration (ng/L)	**Mean survival of F_0 male mysids [%]**	**Mean survival of F0 female mysids [%]**	**Mean survival F0 of mysids [%]**	**Mean survival among F1 mysids following 96 hours [%]**
Control	90	100	81	100
Solvent control	86	94	78	100
Pooled control	88	97	80	100
0.11	74	98	78	95
0.23	88	91	81	100
0.41	71	88	68	100
0.83	85	94	80	98
1.5	81	86	59	95

* = significant difference compared to the pooled control (Fisher's Exact Test with Bonferroni-Holm's Adjustment)

Since no concentration tested resulted in ≥ 50% mortality, the 7, 14, 21 and 28-day LC50 values were empirically estimated to be > 1.5 ng/L, the highest mean measured beta-Cyfluthrin concentration tested.

Table 89: Summary of average total body length and average dry body weight measurements of first generation (F0) male and female mysids measured at the termination of the 28-day life-cycle test exposing mysids (*Americamysis bahia*) to beta-Cyfluthrin

Mean Measured concentration (ng/L)	**Average total body length of male mysids [mm]**	**Average total body length of female mysids [mm]**	**Average dry body weight of male mysids [mg]**	**Average dry body weight of female mysids [mg]**
Control	7.10	7.45	0.80	1.12
Solvent control	7.09	7.41	0.81	1.13
Pooled control	7.09	7.43	0.81	1.13
0.11	6.91	7.40	0.82	1.15
0.23	7.08	7.47	0.90	1.19
0.41	6.88	7.30	0.89	1.13
0.83	6.83	7.17*	0.77	1.03
1.5	6.69	7.14*	0.81	0.92

Table 90: Summary of the first generation (F0) reproductive success (offspring per female) at termination of the 28-day life-cycle exposure of mysids (*Americamysis bahia*)

Mean Measured concentration (ng/L)	Mean number of offspring per female
Control	21.5
Solvent control	23.8
Pooled control	22.6
0.11	21.1
0.23	23.4
0.41	21.5
0.83	15.4*
1.5	11.7*

* = significant difference compared to the pooled control (Fisher's Exact Test with Bonferroni-Holm's Adjustment)

Based on mean measured concentrations of beta-Cyfluthrin, female body length and reproduction (the most sensitive indicators of toxicity), the No-Observed-Effect Concentration (NOEC) was determined to be 0.41 ng/L. The Lowest-Observed-Effect Concentration (LOEC) for mysids was determined to be 0.83 ng/L.

Conclusion:
The 7, 14, 21 and 28-day LC50 values were empirically estimated to be > 1.5 ng/L. The NOEC was determined to be 0.41 ng/L, based on effect on female body length and mean number of offspring per female at the LOEC (0.83 ng/L).

5.4.3 Algae and aquatic plants

Study 1

Author:	Heimbach, F.
Title:	Growth inhibition of green algae (*Scenedesmus subspicatus*) caused by FCR 4545 (techn.)
Date:	27 August 1987
Report no.:	HBF/AL 40
Guidelines:	ISO-Guideline ISO/TC 147/SC 5/WG 5 N 84 (Algal Growth Inhibition Test) from 19.06.84 resp. OECD Guideline No. 201 "OECD-Guideline for Testing of Chemicals", "Alga, Growth Inhibition Test" (07.06.84).
GLP:	Yes
Validity:	Valid

Deviations: The study is valid according to the current OECD Guideline No. 201
Test material: beta-Cyfluthrin techn. (FCR 4545), purity: 98.0%, batch no. 16001/87
Results: The influence of Beta-Cyfluthrin technical (FCR 4545) on growth rate of *Scenedesmus subspicatus* was tested in a static system at one concentration of 0.01 mg a.s./L (nominal). Higher test concentrations could not be examined due to the low water solubility. The test vessels (triplicate) were incubated with an initial number of cells of 10000 for 96 hours in a controlled- environment cabinet with synthetic OECD nutrient medium at 23°C under continuous illumination (8000 lux). The pH was in the range of 7.9 – 8.3. The algal cell density was determined indirectly by photometric measurement of the extinction.
No effects were seen at this concentration (NOEC ≥ 10 µg a.s./L for biomass and the growth rate). Accordingly the EC_{50} is > 10 µg a.s./L based on nominal test concentrations.

Conclusion: NOEC ≥ 10 µg a.s./L; EC_{50} > 10 µg a.s./L

5.4.4 Other aquatic organisms (including sediment)

Study 1

Author:	Kimmel, S.
Title:	Beta-Cyfluthrin: Effects on the Development of Sediment-Dwelling Larvae of *Chironomus riparius* in a Water-Sediment System with Spiked Water
Date:	19 March 2014
Report no.:	D58720
Guidelines:	OECD Guideline No. 219: Sediment-Water Chironomid Toxicity Test Using Spiked Water (adopted 13 April 2004).
GLP:	Yes
Validity:	Valid

Deviations: None

Executive Summary

The purpose of this study was to evaluate effects of beta-Cyfluthrin on the development of sediment dwelling larvae of the midge *Chironomus riparius* in water-sediment systems over 28 days. The test item was applied to the water column in static water-sediment systems. Twenty *Chironomus* larvae (4 collectives of 5 animals per test beaker) per concentration were exposed to 0.1, 0.2, 0.4, 0.8 and 1.6 µg a.s./L nominal concentrations. In addition, 20 *Chironomus* larvae were exposed to a test water control (without test item) and a solvent control (80 µL DMF/L). Four replicates (test beakers) were tested in the biological test at each test concentration, in the control and the solvent control. The test parameters of the study were development time/rate of the midges and the emergence ratio (sum of fully emerged male and female midges divided by the number of larvae introduced into the system). *Chironomus riparius* were observed daily from day 10 to 28 and were fed at least three times per week during the test.

The analytically determined test item concentrations in the application solution samples after application corresponded to 87-92% of the initial nominal test concentrations.

The mean measured concentrations of beta-Cyfluthrin (sum of all isomers) in the water columns after the test item application on day 0 ranged from 25 to 27% of the nominal concentrations in both analysed test concentrations of 0.4 and 1.6 µg a.s./L. The concentrations of beta-Cyfluthrin in the water columns decreased rapidly during the test period, with recovered values down to 3 and 4% on days 1 and 3, and 1% on day 7 after test item application, respectively. At study termination (on day 28), all analytical measured concentrations were found to be below LOQ.

The concentrations found in the pore water samples increased from day 0 to 3 followed by a decrease. On day 0 mean measured concentrations were 0.0188 and 0.0959 µg a.s./L for the test concentrations of nominally 0.4 and 1.6 µg a.s./L, respectively. Throughout the following days, 1, 3 and 7, the mean measured concentration were 0.0479, 0.0771 and 0.0391 µg a.s./L for the nominal concentration of 0.4 µg a.s./L, and 0.123, 0.136 and 0.117 µg a.s./L for the nominal concentration of 1.6 µg a.s./L. At the end of the experiment at day 28 measurement at both concentrations were below LOQ.

In the sediment samples, the concentrations of beta-Cyfluthrin (sum of all isomers) at all evaluated time points and for all analytically measured concentrations were <LOQ.

All reported biological results are related to the nominal initial concentrations of the test item in the water column.

All validity criteria according to the guideline OECD 219 were fulfilled.

Conclusion:
The overall 28-day NOEC of beta-Cyfluthrin for *Chironomus riparius* in this water-sediment study was determined to be 0.4 µg beta-Cyfluthrin/L.
The EC_{10} was determined to be 1.3 µg a.s./L and the EC_{15}, EC_{20} and EC_{50} were all > 1.6 µg a.s./L.
The overall 28-day LOEC was determined to be at the nominal concentration of 0.8 µg beta-Cyfluthrin/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Beta-Cyfluthrin technical
Lot/Batch #:	PNBC000623
Purity:	99.3% w/w
2. Vehicle and/or positive control:	N,N-Dimethylformamide (DMF)
3. Test organisms:	
Species:	Chironomus riparius
Age:	First instars (2-3 days)
Source:	Laboratory bred
Loading:	20 organisms per vessel (600 mL glass beakers)

4. Environmental conditions:

Temperature:	20.2 to 21.1 °C
Photoperiod:	Light/dark 16/8 h
Light intensity:	780 to 985 lux
pH:	8.1 to 8.6
Dissolved oxygen:	7.3 to 9.0 mg O_2/L (= at least 80% oxygen saturation value)
Hardness:	200 mg/L $CaCO_3$

B. STUDY DESIGN

1. Experimental treatments
The effects of beta-Cyfluthrin on the development of sediment-dwelling larvae of the midge *Chironomus riparius* in water-sediment systems were evaluated in a 28 days static toxicity test.
Twenty larvae (4 collectives of 5 animals per test beaker) per concentration were exposed to 0.1, 0.2, 0.4, 0.8 and 1.6 µg a.s./L nominal concentrations. In addition, twenty larvae were exposed to a test water control (without test item) and a solvent control (80 µL DMF/L). Four replicates (test beakers) were tested in the biological test at each test concentration, in the control and the solvent control. First-instar larvae of *Chironomus riparius* were exposed to the test item for 28 days to assess the impact on full maturation of the larvae to adult midges.
Twenty larvae of the first larval stage (2-3 days old) were allocated randomly to each test vessel.
One day after adding the larvae, the test item was applied to the water column of the water-sediment systems (day 0). The test animals were fed at least three times per week.

2. Observations
The emergence and development of *Chironomus riparius* larvae (male and female midges) exposed to beta- cyfluthrin were observed daily from day 10 to 28 .Water temperature, pH and concentration of dissolved oxygen were measured in all test vessels before insertion of the larvae. During the larval

exposure period, these parameters were measured once per week and at study termination.
The water temperature was additionally measured twice per week. Samples for the determination of the concentrations of beta-cyfluthrin in the test medium were taken from all application solutions immediately after the test item application. Further samples (water, pore water and sediment samples) were taken from the water-sediment system on day 0, 1, 3 and 7 for the determination of the test item concentration.

3. Statistical calculations
The mean emergence ratios and development rates of all test concentrations were statistically evaluated on significant differences to the solvent control by the multivariate Williams or Dunnett test for homogenous variances after a one-way analysis of variance (ANOVA). Statistical evaluations were done separately for emerged males and females (development rate) and with pooled sexes (emergence ratio). The mean emergence ratio and development rate of males and females in the control were compared to the solvent control by Student t-tests.
The 28-day EC_{10}, EC_{15}, EC_{20} and EC_{50} of the emergence ratio and the development rates could not be calculated since no toxic effect occurred up to and including the highest tested concentration, except the development rate for male midges, where the EC_{10} value was calculated by means of a Probit analysis using maximum linear likelihood regression.

II. RESULTS AND DISCUSSION

Findings:
Based on the nominal initial test item concentrations of beta-Cyfluthrin, the following results for emergence and development rates after 28 days of exposure were assessed.

Table 91: Results for emergence and development rates for beta-cyfluthrin

Results after 28 days	**Emergence rate (arcsin transformed) of pooled sexes (mg a.s./kg, nominal)**	**Development rate (µg/L, nominal)**	
		Males	**Females**
EC_{10}:	> 1.6	1.3	> 1.6
95% confidence interval:	n.d.	0.93 - 2.0	n.d.
EC_{15}:	> 1.6	> 1.6	> 1.6
95% confidence interval:	n.d.	n.d.	n.d.
EC_{20}:	> 1.6	> 1.6	> 1.6
95% confidence interval:	n.d.	n.d.	n.d.
EC_{50}:	≥1.6	> 1.6	> 1.6
95% confidence interval:	n.d.	n.d.	n.d.
NOEC:	≥1.6	0.4	0.8
LOEC:	> 1.6	0.8	1.6

n.d.: could not be determined due to minor effects of the test item on the development rate

During the test period, the pH values in the test media ranged from 8.1 to 8.6. The dissolved oxygen concentration were at least 7.3 mg/L (= at least 80% oxygen saturation value), and thus sufficiently high throughout the test period. The water temperature varied between 20.2 and 21.1 °C and was thus sufficiently constant. The water temperature differed by less than 1.0 °C between all beakers at any time during the study.

Analytical data: The analytically determined test item concentrations in the application solution samples after application corresponded to 87-92% of the initial nominal test concentrations.

Therefore, all reported biological results are related to the nominal initial concentrations of the test item in the water column. The mean measured concentrations of beta-Cyfluthrin (sum of all isomers) in the water columns after the test item application on day 0 ranged from 25 to 27% of the nominal concentrations in both analysed test concentrations of 0.4 and 1.6 µg a.s./L. The concentrations of beta-Cyfluthrin in the water columns decreased rapidly during the test period, with recovered values down to 3 and 4% on days 1 and 3, and 1% on day 7 after test item application, respectively. At study termination (on day 28), all analytical measured concentrations were found to be below LOQ.
The concentrations found in the pore water samples increased from day 0 to 3 followed by a decrease. On day 0 mean measured concentrations were 0.0188 and 0.0959 µg a.s./L for the lower and higher test concentrations of nominally 0.4 and 1.6 µg a.s./L, respectively. Throughout the following days, 1, 3 and 7, the mean measured concentration were 0.0479, 0.0771 and 0.0391 µg a.s./L for the lower nominal concentration of 0.4 µg a.s./L, and 0.123, 0.136 and 0.117 µg a.s./L for the higher nominal concentration of 1.6 µg a.s./L. At the end of the experiment at day 28 measurement at both concentrations were below LOQ.
In the sediment samples, the concentrations of beta-Cyfluthrin (sum of all isomers) at all evaluated time points and for all analytically measured concentrations were found to be below the limit of quantification.

B. OBSERVATIONS

The emergence ratios per vessel in the control and solvent control ranged from 80 to 100% (thus fulfilling the guideline validity criterion).
Up to and including the highest nominal test concentration of initial 1.6 µg/L, the mean emergence ratios of pooled sexes were not statistically significantly lower than in the control.

Table 92: Emergence ratio (male and female midges pooled)

	Control	**Solvent Control**	**Beta-Cyfluthrin, nominal concentration [µg/L]**				
			0.1	**0.2**	**0.4**	**0.8**	**1.6**
Sum of inserted larvae per treatment	80	80	80	80	80	80	80
Sum of emerged midges per treatment	78	73	67	74	68	61	69
% of emerged midges per treatment (mean)	97.5	91.3	83.8	92.5	85.0	76.3	86.3
Emergence ratio ERarc: Mean	1.490	1.320	1.170	1.300	1.220	1.080	1.240
SD	0.161	0.195	0.150	0.056	0.236	0.182	0.223
Min	1.250	1.110	1.050	1.250	1.050	0-940	1.110
Max	1.570	1.570	1.350	1.350	1.570	1.350	1.570
N	4	4	4	4	4	4	4
% of solvent control	112.9	100.0	88.6	98.5	92.4	81.8	93.9
STAT	n.s*	---	n.s	n.s	n.s	n.s	n.s

ERarc : arcsin-transformed emergence ratio
STAT : results of a Williams t-test ($\alpha = 0.05$, one-sided smaller)
n.s: mean ERarc not statistically significantly lower than in the solvent control
n.s *: mean development rate not statistically significantly lower than in the solvent control (based on a Student t-test, $\alpha = 0.05$, two-sided)

The midges in the control had emerged between days 12 and 21 (and thus fulfilled the validity criterion of the test guideline). Up to and including the tested concentration of initial 0.4 µg a.s./L, the mean development rates of males and females were not statistically significantly lower than in the control. From the nominal concentration of initially 0.8 µg a.s./L on, the mean development rate was slightly, but statistically significantly reduced.

Table 93: Development Rate for Males and Females

Males							
Development rate per treatment (day-1)	**Solvent Control**	**Solvent Control**	**Beta-Cyfluthrin, nominal concentration [µg/L]**				
			0.1	**0.2**	**0.4**	**0.8**	**1.6**
Mean	0.070	0.070	0.070	0.070	0.070	0.070	0.060
SD	0.001	0.001	0.002	0.002	0.004	0.004	0.001
Min	0.070	0.070	0.070	0.070	0.070	0.060	0.060
Max	0.070	0.070	0.070	0.070	0.070	0.070	0.060
N	4	4	4	4	4	4	4
% of solvent control	100.00	100.0	100.0	100.0	100.0	100.0	85.7
STAT	n.s*	---	n.s	n.s	n.s	s.	s.
Females							
Development rate per treatment (day-1)	**Solvent Control**	**Control**	**Beta-Cyfluthrin, nominal concentration [mg/kg L]**				
			0.1	**0.2**	**0.4**	**0.8**	**1.6**
Mean	0.060	0.060	0.060	0.060	0.060	0.060	0.060
SD	0.003	0.003	0.006	0.001	0.002	0.001	0.004
Min	0.060	0.060	0.050	0.060	0.060	0.060	0.050
Max	0.060	0.070	0.070	0.060	0.060	0.060	0.060
N	4	4	4	4	4	4	4
% of solvent control	100.0	100.0	100.0	100.0	100.0	100.0	100.0
STAT	n.s*	---	n.s	n.s	n.s	n.s	s.

STAT : results of a Williams t-test (males) or Dunnett t-test (females, $\alpha = 0.05$, one-sided smaller)
n.s: mean development rate not statistically significantly lower than in the solvent control
s.: mean development rate statistically significantly lower than in the solvent control
n.s *: mean development rate not statistically significantly lower than in the solvent control (based on a Student t-test, $\alpha = 0.05$, two-sided)

No symptoms of toxicity were observed at the larvae, pupae and emerged midges during the study.

III. CONCLUSION

The overall 28-day NOEC of beta-Cyfluthrin for *Chironomus riparius* in this water-sediment study was determined to be 0.4 µg beta-Cyfluthrin/L. The EC_{10} was determined to be 1.3 µg a.s./L and the EC_{15}, EC_{20} and EC_{50} were all > 1.6 µg a.s./L.
The overall 28-day LOEC was determined to be at the nominal concentration of 0.8 µg beta-Cyfluthrin/ L.

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

Beta-cyfluthrin produces acute $L(E)C_{50}$ values in concentrations > 0.01 mg/L for algae, > 0.0000001 ≤ 0.0001 mg/L for invertebrates and > 0.00001 ≤ 0.001 mg/L for fish. Chronic NOEC values in concentrations > 0.0000001 ≤ 0.000001 mg/L for aquatic invertebrates, > 0.000001 ≤ 0.00001 mg/L for fish and ≤ 0.01 mg/L for algae were determined.

The results of the test on the biodegradation of beta-cyfluthrin in the water/sediment system and abiotic degradation show that beta-cyfluthrin is considered not rapidly degradable (degradation < 70 % within 28 days) for purposes of classification and labelling.

Beta-cyfluthrin (Isomers II and IV) has a log $K_{o/w}$ of 5.8 -5.9 (25°C). The experimentally derived kinetic BCF of 1822 for beta-cyfluthrin related to total radioactive residues and whole fish is above the trigger of 500 (criterion for bioaccumulation potential conform Regulation EC 1272/2008).

CLP- Acute aquatic hazards

According to the criteria of the CLP Regulation, a substance is classified for aquatic acute toxicity if in an aquatic acute toxicity study, an $L(E)C_{50}$ of ≤ 1 mg/l is obtained for any of the three trophic levels fish, invertebrates and algae/aquatic plants.

The lowest $L(E)C_{50}$ obtained for beta-cyfluthrin are 0.00000055, 0.000068 and > 0.01 mg/L in invertebrates (*Hyalella azteca*), fish and algae, respectively. Beta-cyfluthrin therefore fulfils the criteria for classification as Aquatic Acute Cat. 1 (H400).

An M-factor of 1000 000 for acute toxicity is proposed based on $L(E)C_{50}$ value of 0.00000055 mg/L in aquatic invertebrates. (0.0000001 < $L(E)C_{50}$ ≤ 0.000001 mg/L)

CLP - Aquatic chronic hazards

According to the criteria of the 2nd ATP to the CLP Regulation, when NOEC values are available for all trophic levels, a substance is classified for aquatic chronic hazards if a NOEC or EC_{10} of ≤ 1 mg/L is obtained in a long-term aquatic toxicity study. The assignment of a hazard category depends on the NOEC value and whether the substance is rapidly degradable or not.

Beta-cyfluthrin is considered to be not rapidly degradable (see section 5.1.3). NOEC values for beta-cyfluthrin are available for all trophic levels. The lowest NOEC (28 d) is 0.00000041 mg/L for aquatic invertebrates (*Americamysis bahia*). Beta-cyfluthrin therefore fulfils criteria for classification as Aquatic Chronic Cat.1 (H410).

An M-factor of 100 000 for chronic toxicity is proposed based on the NOEC (28d) value of 0.41 ng/L for aquatic invertebrates. (0.0000001 < NOEC ≤ 0.000001 mg/L).

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

Beta-cyfluthrin fulfils the criteria for classification as Aquatic Acute 1 (H400) with an acute M-factor of 1000 000.

Beta-cyfluthrin fulfils the criteria for classification as Aquatic Chronic 1 (H410) with a chronic M-factor of 100 000.

Labelling:

Signal word: Warning

Pictogram: GHS09

Hazard statement: H410 (Very toxic to aquatic life with long lasting effects)

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Current entry in Annex VI, CLP Regulation: Aquatic Acute 1 (H400), Aquatic Chronic 1 (H410).

DS proposal: Aquatic Acute 1 (H400), M-factor=1 000 000, Aquatic Chronic 1 (H410), M-factor=100 000

Degradation

Abiotic degradation

Hydrolysis of beta-cyfluthrin was studied as a function of pH on mixtures of four different diastereomers of identical composition forming mixtures during the hydrolysis. The hydrolysis half-lives were calculated for temperatures 20 °C and 25 °C (by extrapolation).

Beta-cyfluthrin is found to be stable at pH 4 (> 1 year), as well as relatively stable at pH 7 (DT_{50} 61 - 281 days). The hydrolysis rates increase at pH 9 (DT_{50} < 2 days). These half-lives were recalculated without experiment showing same results.

Direct photodegradation half-lives were calculated based on a mean quantum yield of 0.001149 and the molar extinction coefficients. Half-lives between 4.97 days (summer 30-50° latitude) and 93.5 days (winter 60° latitude) were estimated, dependent on degree of latitude and seasonal conditions; half-lives ranged from 5.2 to 56 days. Weak potential for direct photolytic interactions of beta-cyfluthrin with sunlight in the environment is shown. Moderate photo-degradation of beta-cyfluthrin in aqueous solution in a range of 19 to 21% was measured by HPLC after a maximum irradiation period of 500 minutes.

Biodegradation

No data is available for ready biodegradation.

The dissipation of beta-cyfluthrin was studied in two different water-sediment systems and later evaluated according to FOCUS 2006 where beta-cyfluthrin was shown not rapidly degradable with a DT_{50} of 14.4 – 53 days (total system) and DT_{50} of 14.3 – 81.5 days (sediment).

Beta-cyfluthrin is hydrolytically stable under acidic and neutral conditions. Aquatic photolysis is not considered an important transformation route for beta-cyfluthrin in the environment with DT_{50} of 5 - 56 days.

The metabolism/degradation pathway of beta-cyfluthrin is expected via cleavage of the ester or diphenyl ether bond hydroxylation at the phenoxy ring and hydrolysis of the cyano group. Further degradation mainly resulted in generation of CO_2 and bound residues.

The DS gave no information on the hazards presented to the aquatic environment by the metabolites.

Metabolites of beta-cyfluthrin:

Environmental compartment	Metabolites
Biodegradation	
Water sediment	1. FPB-acid (4-fluoro-3-phenoxybenzoic acid, FCR 3191, CAS-no.: 77279-89-1) Water: 29 % of applied radioactivity 2. FPB-ald Sediment: 16 % of applied radioactivity

The DS concluded that based on the available information, beta-cyfluthrin does not fulfil the criteria to be considered as rapidly degradable in the aquatic environment. This is based on data for beta-cyfluthrin.

Bioaccumulation

Beta-cyfluthrin consists mainly of two diastereoisomers II and IV, with log K_{OW} values of 5.91 for diastereoisomer IV and 5.94 for diastereoisomer II. An approximate estimation of the bioconcentration factor BCF_{fish} has been performed for the diastereoisomers using the standard equation in the EU Technical Guidance Document (TGD) on Risk Assessment (2003), Part II, 3.8.3.2. The calculated BCF is ranging between 22336 L/kg_{wet} in fish, for diastereoisomer II, and 21062 L/kg_{wet} in fish, for diastereoisomer IV.

Results of a bioaccumulation study with Bluegill sunfish at an average exposure concentration of 0.12 µg eq/L are given for beta-cyfluthrin. The plateau level, determined as the average concentration in the fish of the last two time intervals of the uptake phase, was 0.155 µg/g. Due to a malfunction in the flow system, no reliable steady state in uptake phase was reached and the BCFss is not reliable. The lipid and growth-normalised kinetic BCF [L kg^{-1}] BCF_{kLg} is 1822 for beta-cyfluthrin related to total radioactive residues and this whole fish value is above the CLP criterion of 500.

The DS considers beta-cyfluthrin as having a high potential for bioaccumulation based on reliable data for diastereomers and beta-cyfluthrin.

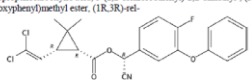
Justification of read-across of data from cyfluthrin to beta-cyfluthrin for aquatic toxicity

Beta-cyfluthrin and cyfluthrin are mixtures of eight isomers four of the isomers are considered active. The proportion of diastereoisomer pairs and their structures in beta-cyfluthrin and cyfluthrin are shown in figures below:

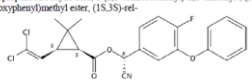
Diastereomer	Cyfluthrin	Beta-Cyfluthrin
I (1R-3R-R+1S-3S-S = 1:1;cis) CAS: 86560-92-1	23-27 %	< 2 %
II **(1R-3R-S + 1S-3S-R = 1:1, cis)** **CAS: 86560-93-2**	**17 -21 % (mean 19 %)**	**30-40 % (mean 35 %)**
III (1R-3R-R + 1S-3R-S = 1:1;trans) CAS: 86560-93-2	32-36 %	< 3%
IV **(1R-3S-S + 1S-3R-R = 1:1; trans)** **CAS:** **CAS: 86560-95-4**	**21-25 % (mean 22%)**	**57-67 % (mean 62 %)**
Sum of active diastereoisomers	~ 41 %	~ 97 %
Relation of II/IV	0,86	0,56

Active diastereoisomers are written in **bold**.

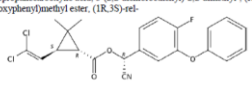
Diastereomer I: CAS No 86560-92-1
Cyclopropanecarboxylic acid, 3-(2,2-dichloroethenyl)-2,2-dimethyl-, (R)-cyano(4-fluoro-3-phenoxyphenyl)methyl ester, (1R,3R)-rel-



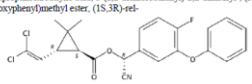
Diastereomer II: CAS No 86560-93-2
Cyclopropanecarboxylic acid, 3-(2,2-dichloroethenyl)-2,2-dimethyl-, (R)-cyano(4-fluoro-3-phenoxyphenyl)methyl ester, (1S,3S)-rel-



Diastereomer III: CAS No 86560-94-3
Cyclopropanecarboxylic acid, 3-(2,2-dichloroethenyl)-2,2-dimethyl-, (R)-cyano(4-fluoro-3-phenoxyphenyl)methyl ester, (1R,3S)-rel-



Diastereomer IV: CAS No 86560-95-4
Cyclopropanecarboxylic acid, 3-(2,2-dichloroethenyl)-2,2-dimethyl-, (R)-cyano(4-fluoro-3-phenoxyphenyl)methyl ester, (1S,3R)-rel-



Beta-cyfluthrin represent a major constituent of cyfluthrin. Beta-cyfluthrin and cyfluthrin share the same chemical structure, consisting of three asymmetric carbon atoms. These lead to four diastereomers, each consisting of an enantiomer pair. While cyfluthrin consists of all four diastereomers (referred to as diastereomer I, II (1R,3R, αS + 1S,3S, αR = 1:1; cis), III and IV (1R,3S, αS + 1S,3R αR =1:1; trans)), beta-cyfluthrin mainly consists of the two most active diastereomers II and IV (II: 30.0 – 40.0 %, IV: 57.0 – 67.0 % of the sum of the four diastereoisomers). Due to the common structure of the diastereomers it can be assumed that all diastereomers show a similar chemical and biological activity and share the same insecticidal mode of action. It was generally accepted for the biocidal (cyfluthrin, ECHA 2018) and plant protection evaluation (beta-cyfluthrin, EFSA 2002) that both substances share a similar toxicological profile.

There are indications from the scientific literature (Leicht, 1996) that diastereomers I and III could be regarded as around one order of magnitude less active than isomers II and IV. If only diastereomers II and IV would be biologically active, beta-cyfluthrin would be approximately 2.4 times more toxic as cyfluthrin (cyfluthrin consists of 40% diastereomers II + IV). However, it has been assumed that diastereomers I and III also show significant biological activity and a significant degree of isomerisation between the diastereomers in the environment or in organisms. Furthermore, it has been shown that isomer III can synergise the activity of isomer IV. Consequently, an activity ratio of 1.3 between cyfluthrin and beta-cyfluthrin has been postulated, instead of the expected value of 2.4 based on the 40% beta-cyfluthrin content of cyfluthrin (Leicht, 1996).

Generally, it can be expected that beta-cyfluthrin is at least equally toxic as cyfluthrin to aquatic organisms. Therefore, equivalent level of relevance of data for both substances can be concluded and effect studies with cyfluthrin are considered for aquatic toxicity in the hazard assessment of beta-cyfluthrin.

Aquatic toxicity

Summary of relevant information on aquatic toxicity of beta-cyfluthrin

Test details	Test species	Result µg/L	Reference
Fish			
beta-cyfluthrin OECD TG 203 flow-through, 96 h	*Oncorhynchus mykiss*	LC_{50} = 0.089 based on mean measured concentrations	Anonymous-F1,1988a (Rep. No. FF-207)
beta-cyfluthrin OECD TG 203 flow-through, 96 h	*Oncorhynchus mykiss*	LC_{50} = 0.068 based on mean measured concentrations	Anonymous-F2, 1994a (Rep. No 103231)
beta-cyfluthrin OECD TG 203 flow-through, 96 h	*Lepomis macrochirus*	LC_{50} = 0.28 based on mean measured concentrations	Anonymous-F3, 1994b (Rep. No 103232)
beta-cyfluthrin OECD TG 203 flow-through, 96 h	*Leuciscus idus melanotus*	LC_{50} = 0.33 based on mean measured concentrations	Anonymous-F4, 1988b (Rep. No. FO-1011)
cyfluthrin (purity 96%) Test laboratory's internal method, equivalent to EPA - FIFRA § 72-4 and OECD TG 210 flow-through, 58 d	*Oncorhynchus mykiss*	NOEC = 0.01 based on mean measured concentrations	Anonymous-F5, 1985 (Rep. No. 683)
^{14}C-cyfluthrin (purity 99%) US-EPA FIFRA § 72-4 guideline, 40 CFR, Section 158.145 flow-through, 307 d	*Pimephales promelas*	NOEC = 0.14 based on mean measured concentrations	Anonymous-F6, 1990 (Rep. No. 100097)
Invertebrates			
beta-cyfluthrin OECD TG 202 semi-static, 48h	*Daphnia magna*	LC_{50} = 0.105 based on mean measured concentrations	Kimmel, 2014a (Rep. No. D58707)
beta-cyfluthrin EPA-FIFRA §72-3 flow-through, 96 h	*Americamysis bahia*	LC_{50} = 0.0022 based on mean measured concentrations	Machado, 1994 (Rep. No. 106797)
cyfluthrin (purity 95.8%) OCSPP draft 850.1020 flow-through, 96 h	*Hyalella azteca*	**LC_{50} = 0.00055** based on mean measured concentrations	Bradley, 2013 (Rep. No. 13656.6168)
beta-cyfluthrin OECD TG 211 semi-static, 21 d	*Daphnia magna*	NOEC = 0.025 (offspring production, parental body length) based on mean measured concentrations	Kimmel, 2014b (Rep. No. D58718)
beta-cyfluthrin (purity 99.2%) OCSPP draft 850.1350 flow-through, 28 d	*Americamysis bahia*	**NOEC = 0.00041** based on mean measured concentrations	Schwader, 2013 (Rep. No. 13798.6307)

Algae and other aquatic organisms			
beta-cyfluthrin (purity 98.0%) OECD TG 201 static, 96 h	*Scenedesmus subspicatus*	EC_{50} > 0.01 mg/L based on nominal test concentrations	Heimbach, 1987 (Rep. No HBF/AL 40)
beta-cyfluthrin OECD TG 219 static, spiked water, 28 d	*Chironomus riparius*	NOEC = 0.4 based on nominal concentrations	Kimmel, 2014c (Rep. No. D58720)

Acute Aquatic Toxicity

Acute toxicity of beta-cyfluthrin to fish was investigated in studies, which can be considered valid and equivalent to OECD TG 203. Three different fish species (Rainbow trout, Bluegill sunfish and Golden orfe) were exposed under flow-through conditions for 96 h. The LC_{50} values ranged from 0.068 µg/L to 0.33 µg/L. The lowest no observed effect level out of the four acute fish toxicity tests was 0.068 µg/L. The results are based on mean measured concentrations.

Two acute invertebrate tests are given for beta-cyfluthrin with Daphnia and mysid shrimp based on mean measured concentrations and considered valid. The 48-h EC_{50} for *Daphnia magna* exposed to beta-cyfluthrin was 0.105 µg/L. No effect on immobilisation was reported up to 0.05 µg/L. The *A. bahia* 96-h LC_{50} was 0.0022 µg/L and NOEC of 0.0013 µg/L. The lowest value for invertebrates is based on cyfluthrin with *H. azteca*, the 96-h study, based on mortality, a **LC_{50} = 0.00055 µg/L** was derived.

Chronic Aquatic Toxicity

The chronic toxicity information for beta-cyfluthrin is based on cyfluthrin data in two fish species: Rainbow trout and Fathead minnow. Toxicity was investigated according to a flow-through test procedure, which can be considered equivalent to EPA-FIFRA G. 72-4 and OECD TG 210. The NOEC values were determined to be 0.01 µg/L and 0.14 µg/L based on mean measured concentrations.

The presented valid Daphnia chronic study was conducted in accordance with OECD TG 211. The 21-day NOEC value without effects after exposure period was 0.025 µg/L based on mean measured concentrations for *Daphnia magna*. The chronic study with beta-cyfluthrin in *A. bahia* covers 28 days under flow-through conditions and has been performed with five test concentrations of nominally 0.25, 0.50, 0.99, 2.0 and 4.0 ng/L (corresponding to mean measured concentrations of 0.11, 0.23, 0.41, 0.83 and 1.5 ng/L) using seawater. The study is considered as valid and reliable. Based on female body length and reproduction (mean number of offspring), a **NOEC of 0.00041 µg/L** beta-cyfluthrin (mean measured) was derived.

Toxicity in Algae

The overall 28-d NOEC of beta-cyfluthrin for *C. riparius* in this water-sediment study was determined to be 0.4 µg/L. For green algae, no effects were seen, giving a NOEC ≥ 0.01 mg/L for biomass and the growth rate.

Comments received during public consultation

Three MSCAs supported the proposed environmental classification Aquatic acute 1 (H400), M = 1000000 and Aquatic Chronic 1 (H410), M = 100000 based on the available data for the most sensitive species (Invertebrates: *H. Azteca*; 96-h LC_{50}= 0.55 ng/L as a read-across from cyfluthrin; and *A. bahia* 28-d NOEC=0.41 ng/L beta-cyfluthrin, respectively).

However, one of the MSCAs stated that chronic classification could be based on acute *H. azteca* data with cyfluthrin as no chronic data is available for this species and the surrogate would result in a more stringent chronic classification (Aquatic Chronic 1, M = 1000000).

Two MSCAs pointed out the difference in the composition of beta-cyfluthrin and cyfluthrin as well as the importance to take into account the different biological activity and therefore the sensitivity of the biologically active isomers towards different species (algae, fish, and invertebrates). The DS responded that this had been taken into account in their assessment in the CLH report.

Assessment and comparison with the classification criteria

Taking into account the grounds for read-across of aquatic toxicity data from cyfluthrin to beta-cyfluthrin based on beta-cyfluthrin consisting of up to 97% of the sum of active diastereomers (II + IV) and sharing the same chemical structure with cyfluthrin, RAC agrees to the use of data read-across from cyfluthrin for aquatic toxicity endpoints.

RAC agrees with the DS submitter that based on data for beta-cyfluthrin, there is no evidence that beta-cyfluthrin degrades to a degree greater than 70% over 28 days. Therefore, RAC agrees with the DS to consider beta-cyfluthrin as not rapidly degradable.

RAC also agrees that the available information indicates that the experimentally determined BCF for beta-cyfluthrin exceeds the trigger value of 500, which is supported by the available Log K_{ow} values for the diastereomers. Consequently, RAC agrees with the DS that beta-cyfluthrin is bioaccumulative in the aquatic environment.

There is reliable experimental data on acute toxicity on fish, invertebrates, and algae available with the lowest value being for beta-cyfluthrin with *A. bahia* 96-h LC_{50} = 0.0022 µg/L and a 96-h LC_{50} for *H. azteca* of 0.00055 µg/L as read-across from cyfluthrin. RAC notes that the reliability given to the algae study with *S. subspicatus* is questionable as no analytical monitoring was reported, while the substance has a high adsorption potential. However, RAC accepts the study is suitable for CLP and that its inclusion does not affect the classification outcome.

RAC agrees with the DS that based on the content of the biologically active isomers, beta-cyfluthrin is possibly up to 2.4 or 1.3 times more toxic than cyfluthrin and notes that the possible effects on the most sensitive species *H. azteca* need to be considered with regards to aquatic hazard classification.

There is no chronic data available for beta-cyfluthrin in fish. There are reliable experimental data on chronic toxicity for fish based on cyfluthrin and, following the acceptance of the read-across, these data are used to complete the data set. Reliable beta-cyfluthrin data is available for invertebrates and algae.

The lowest value is a 28-d NOEC for *A. bahia* 0.00041 µg/L based on a study with beta-cyfluthrin resulting in classification of beta-cyfluthrin as Aquatic Chronic 1, H410 with M-factor

100 000. However, classification based on beta-cyfluthrin chronic data as proposed by the DS may underestimate the chronic effects of beta-cyfluthrin on the most sensitive species (*H. Azteca*) as well as its possible toxic potency. Even though the available chronic data does not indicate which species is in fact more sensitive for beta-cyfluthrin (*A. bahia* or *H. azteca*), using the surrogate approach based on the acute data, read-across from cyfluthrin would result in a more stringent chronic classification. All data RAC considers for comparison with the CLP criteria are summarised in the table below.

Table: *Summary of data for classification of beta-cyfluthrin*

Results	**Test substance**	**Remarks**
Fish		
96-h LC_{50} = 0.000068 mg/L *Onchorhynchus mykiss*	beta-cyfluthrin	
58-d NOEC = 0.000010 mg/L *Oncorhynchus mykiss*	cyfluthrin	growth, read-across from cyfluthrin
Invertebrates		
96-h LC_{50} = 0.00000055 mg/L *Hyalella azteca*	cyfluthrin	read-across from cyfluthrin
28-d NOEC = 0.00000041 mg/L *Americamysis bahia*	beta-cyfluthrin	reproduction, parental body length
Algae/Aquatic plants		
E_rC_{50} = > 10 µg/L NOE_rC = 10 µg/L *Scenedesmus subspicatus*	beta-cyfluthrin	

In conclusion, RAC agrees with the DS that considering the available acute data for beta-cyfluthrin (which would result in an aquatic acute classification of Aquatic Acute 1, M=100000), the classification should be based on read-across from cyfluthrin. Based on this data, beta-cyfluthrin warrants classification as **Aquatic Acute 1 (H400), M=1000000** (96-h LC_{50} for *H. azteca* of 0.00055 µg/L as read-across from cyfluthrin).

For chronic aquatic hazards, RAC disagrees with the DS's proposal and proposes to classify beta-cyfluthrin as **Aquatic Chronic 1 (H410), M=1000000** based on the surrogate approach using acute data (96-h LC_{50} for *H. azteca* of 0.00055 µg/L) read-across from cyfluthrin.

RAC notes that if data becomes available for beta-cyfluthrin with *H. Azteca*, the classification of beta-cyfluthrin could be reconsidered.

6 OTHER INFORMATION

None.

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8 ANNEXES

Confidential Annex