Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

cyfluthrin (ISO); α-cyano-4-fluoro-3-phenoxybenzyl-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate

EC Number: 269-855-7
CAS Number: 68359-37-5

CLH-O-0000006802-74-01/F

Adopted

4 May 2020

4 May 2020

CLH-O-0000006802-74-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **cyfluthrin (ISO); α-cyano-4-fluoro-3-phenoxybenzyl-3-(2,2-dichlorovinyl)-2, 2-dimethylcyclopropanecarboxylate**

EC Number: **269-855-7**

CAS Number: **68359-37-5**

The proposal was submitted by **Germany** and received by RAC on **28 November 2018.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Germany has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **18 March 2019**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **24 May 2019**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Michal Martínek**

Co-Rapporteur, appointed by RAC: **Raili Moldov**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **4 May 2020** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	607-253-00-1	cyfluthrin (ISO); α-cyano-4-fluoro-3-phenoxy-benzyl-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate	269-855-7	68359-37-5	Acute Tox. 3* Acute Tox. 2* Aquatic Acute 1 Aquatic Chronic 1	H331 H300 H400 H410	GHS06 GHS09 Dgr	H331 H300 H410		M = 1000	
Dossier submitters proposal	607-253-00-1	cyfluthrin (ISO); α-cyano-4-fluoro-3-phenoxybenzyl-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate	269-855-7	68359-37-5	**Retain** Aquatic Acute 1 Aquatic Chronic 1 **Add** Lact. STOT SE 3 **Modify** Acute Tox. 2 Acute Tox. 2	**Retain** H300 H400 H410 **Add** H362 H335 **Modify** H330	**Retain** GHS06 GHS09 Dgr	**Retain** H300 H410 **Add** H362 H335 **Modify** H330		**Add** inhalation: ATE = 0.081 mg/L (dusts or mists) oral: ATE = 14.3 mg/kg bw M = 100000 (chronic) **Modify** M = 1000000 (acute)	
RAC opinion	607-253-00-1	cyfluthrin (ISO); α-cyano-4-fluoro-3-phenoxybenzyl-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate	269-855-7	68359-37-5	**Retain** Aquatic Acute 1 Aquatic Chronic 1 **Add** Lact. STOT SE 1 **Modify** Acute Tox. 2 Acute Tox. 2	**Retain** H300 H400 H410 **Add** H362 H370 (nervous system) **Modify** H330	**Retain** GHS06 GHS09 Dgr **Add** GHS08	**Retain** H300 H410 **Add** H362 H370 (nervous system) **Modify** H330		**Add** inhalation: ATE = 0.14 mg/L (dusts or mists) oral: ATE = 14 mg/kg bw M = 1000000 (chronic) **Modify** M = 1000000 (acute)	
Resulting Annex VI entry if agreed by COM	607-253-00-1	cyfluthrin (ISO); α-cyano-4-fluoro-3-phenoxybenzyl-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate	269-855-7	68359-37-5	Lact. Acute Tox. 2 Acute Tox. 2 STOT SE 1 Aquatic Acute 1 Aquatic Chronic 1	H362 H330 H300 H370 (nervous system) H400 H410	GHS06 GHS08 GHS09 Dgr	H362 H300 H370 (nervous system) H330 H410		inhalation: ATE = 0.14 mg/L (dusts or mists) oral: ATE = 14 mg/kg bw M = 1000000 M = 1000000	

GROUNDS FOR ADOPTION OF THE OPINION

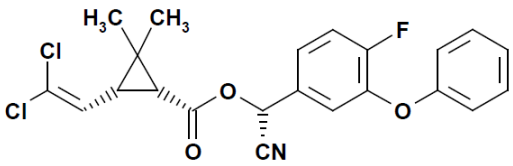
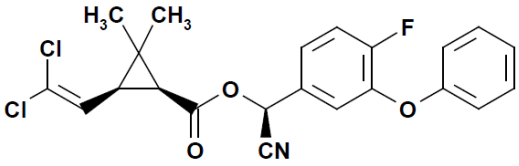
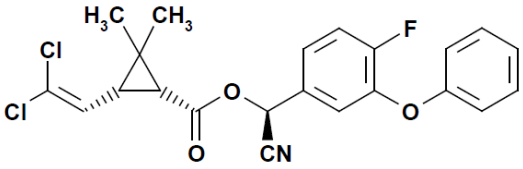
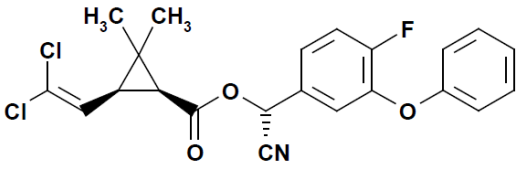
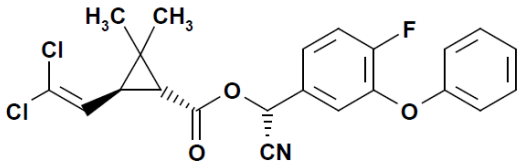
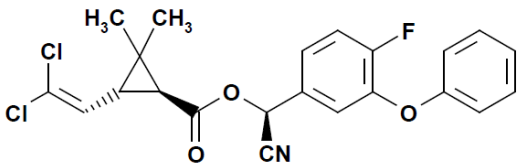
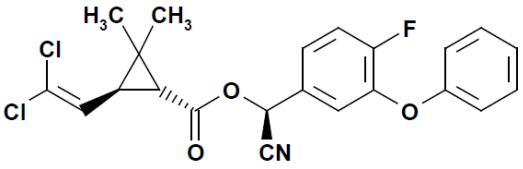
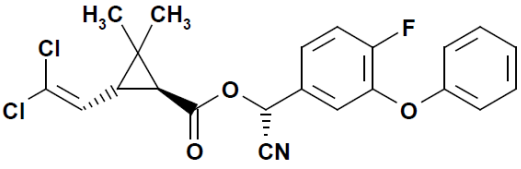
RAC general comment

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 between the two substances (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.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

A flash point of 131°C was determined according to the standard DIN EN ISO 2719 (Council Directive 67/548/EEC, Annex V, A.9).

Experience in handling and use indicates cyfluthrin is not pyrophoric and does not react with water to liberate flammable gases.

Furthermore, it was also tested in a standard auto-ignition temperature study (EEC Method A.15) and spontaneous ignition was found at 375°C.

Cyfluthrin has no oxidising properties according to EEC Method A.21 and no explosive properties according to of EEC Method A.14.

The DS also proposed no classification for the physical hazard classes listed below with the accompanied rationale:

Self-reactive substances and mixtures/explosive

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

Pyrophoric solids

Cyfluthrin is known to be stable in contact with air at room temperature for prolonged periods of time (days) 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 does not need to be applied because the organic substance does not contain metals or metalloids.

Oxidising solids

No classification is warranted because the organic substance contains oxygen, chlorine, and fluorine atoms that are chemically bonded only to carbon or hydrogen and hence, the classification procedure does not need to be applied.

Organic peroxides

No classification is warranted because 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).

The DS concluded that cyfluthrin should not be classified as flammable, oxidising or explosive or for any other physical hazard classes.

Comments received during public consultation

No comments received during public consultation.

Assessment and comparison with the classification criteria

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 and the chemical structure does not raise a concern about corrosive properties (no dissociation etc.), no classification is warranted.

RAC agrees with the rationale of the DS that **no classification with regards to the physical hazards is warranted for cyfluthrin**.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute oral toxicity

The DS summarised data from 20 acute oral toxicity studies with cyfluthrin and six with beta-cyfluthrin. The acute oral toxicity of cyfluthrin depends on the vehicle, with Cremophor/water leading to the lowest LD_{50} values, down to 14.3 mg/kg bw for cyfluthrin (study 5). The lowest LD_{50} included in the CLH dossier for beta-cyfluthrin was 77 mg/kg bw from a study employing acetone/peanut oil as a vehicle (study 22), but the DS was not aware of any acute oral toxicity study with beta-cyfluthrin using Cremophor. The DS proposed classification as Acute Tox. 2 based on an LD_{50} of 14.3 mg/kg bw (study 5).

Acute dermal toxicity

Three acute dermal toxicity studies were available for cyfluthrin, all reporting LD_{50} values of >5000 mg/kg bw. However, these studies were considered supplementary by the DS due to insufficient reporting. The DS proposed no classification for acute dermal toxicity based on a study with beta-cyfluthrin reporting an LD_{50} of >2000 mg/kg bw (study 42).

Acute inhalation toxicity

The DS summarised data from eight acute inhalation toxicity studies with cyfluthrin and two studies with beta-cyfluthrin. The DS proposed classification for cyfluthrin as 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 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.

Assessment and comparison with the classification criteria

Acute oral toxicity

Out of the vehicles tested, aqueous Cremophor consistently yielded the lowest LD_{50} values for both cyfluthrin and beta-cyfluthrin. The rat LD_{50} values for cyfluthrin in Cremophor ranged between 14.3 and 20 mg/kg bw compared to ca. 160-250 mg/kg bw in acetone/oil. Other vehicles (including PEG 400) led to higher LD_{50} values.

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 cyfluthrin for Acute Tox. 2; H300 with an ATE of 14 mg/kg bw** based on a rat acute toxicity study with cyfluthrin using aqueous Cremophor as a vehicle (study 5).

Acute dermal toxicity

Three acute dermal toxicity studies are available for cyfluthrin (studies 37, 38, 39; rat, vehicle Cremophor/water, PEG 400 or NaCl solution), all from the same author and all giving LD_{50} values of >5000 mg/kg bw. The available information on these studies is not very detailed.

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} in a standard acute study with cyfluthrin was 0.14 mg/L (study 33; mouse, head/nose only, vehicle ethanol/PEG 400; OECD TG 403, GLP). This LC_{50} value corresponds to Category 2 ($0.05 < ATE \leq 0.5$ mg/L). Thus, **RAC proposes to classify cyfluthrin as Acute Tox. 2; H330 with an ATE of 0.14 mg/L (dusts or mists).**

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 toxicity 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. Still, 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 = 14 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 = 0.14 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.

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 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 based on the guideline study 49 with additional support of the non-guideline study 47.

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 based on the guideline-compliant *in vivo* studies with beta-cyfluthrin (study 50 and 51).

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

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

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 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 based on a negative GPMT with cyfluthrin (study 57) and a negative Buehler test with beta-cyfluthrin (study 58).

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**.

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 receive 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 the 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. Statistical evaluation: post-implantation loss 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)

Two-generation study with cyfluthrin and DNT study with beta-cyfluthrin (studies 70 and 80)

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.

Rat PNDT studies via inhalation with cyfluthrin (studies 77 and 78)

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/m^3. 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, which indicates a relatively high background incidence, 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 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 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	0/25	0/26	19/26*	9/25*

occurrence]			[PND 7–16]	[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 2-generation study (study 70) attributable to transfer of the test substance via milk and occurring in the absence of maternal toxicity.

ENVIRONMENTAL HAZARD EVALUATION

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), M = 1000

DS proposal: Aquatic Acute 1 (H400), M = 1000000, Aquatic Chronic 1 (H410), M = 100000

Degradation

Abiotic degradation

Hydrolysis of cyfluthrin has been 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) and later recalculated for 12°C for fresh water.

Cyfluthrin is found to be stable at pH 4 and 5 (> 2 years), as well as relatively stable at pH 7 (DT_{50} 193 - 270 days – the maximum value corresponding to 512 d at 12°C). The hydrolysis rates increase at pH 9 (DT_{50} < 2 days), mean half-life of around 2.6 days was calculated to 12°C.

Direct photodegradation half-lives were calculated based on a reaction quantum yield of 0.0052 and UV absorption data. Using GC-solar, half-lives between 2.8 days (summer 30-50° latitude) and 58 days (winter 60° latitude) were estimated, being dependent on degree of latitude and seasonal conditions; half-lives ranged from 2.8 to 32 days. Consequently, solar radiation is regarded to contribute to the degradation of cyfluthrin in aquatic systems.

The photodecomposition of cyfluthrin on sandy loam by natural sunlight at a concentration of 37 mg a.i./kg soil, for up to 6 days, followed a biphasic degradation pattern and showed ready degradation. A recalculated half-life value (4.4 days at mean 25 °C) corresponds to DT_{50} 12.3 days at 12 °C.

Photolysis of cyfluthrin in the natural sunlight results in DT_{50} <1 day and during mercury light exposure DT_{50} 12.2 days.

Biodegradation

No ready biodegradation screening test was available for cyfluthrin.

Cyfluthrin was shown to dissipate rapidly in surface water under aerobic aquatic conditions with a non-adapted inoculum during the first days of incubation with a DT_{50} (25°C) of 6.3 days, corresponding to DT_{50} 17.8 days at 12°C. Dissipation clearly decreased after day 7 due to sorption to colloids increased by turbidity and the microbial count with incubation time. No mineralisation was observed.

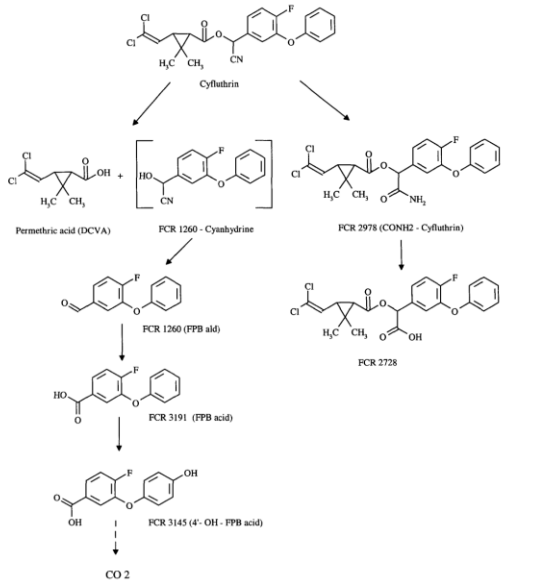
The dissipation of cyfluthrin was studied in four water-sediment systems where cyfluthrin was transferred rapidly from the water to the sediment and degraded in all systems. DT_{50} values < 10 days at 12°C were determined.

The metabolism/degradation pathway of cyfluthrin is mainly 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 of the metabolites to the aquatic environment. However, the metabolite FPB-ald

(4-fluoro-3-phenoxybenzaldehyde, FCR 1260, CAS-no.: 68359-57-9) appears to be classified as toxic to aquatic life with long-lasting effects (H411) according to Annex VI of the CLP Regulation.

Metabolites of cyfluthrin:

Environmental compartment	**Metabolites**
Abiotic degradation	
Hydrolysis	• FPB-ald (4-fluoro-3-phenoxybenzaldehyde, FCR 1260, CAS-no.: 68359-57-9) up to 89% of the radioactivity at pH 9 (day 21) up to 11% at pH 7 (day 35). *FPB-ald was stable to hydrolysis.* • Permethric acid ((DCVA) 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid, CAS-no.: 55701-05-8) *Also considered stable (DT_{50} < 1 years) during hydrolysis.*
Photolysis	• FPB-ald Natural sunlight: max. 18% Mercury light: max. 3% • FPB-acid (4-fluoro-3-phenoxybenzoic acid, FCR 3191, CAS-no.: 77279-89-1) Natural sunlight: max. 37% Mercury light: max. 8.5%
Biodegradation	
Aerobic aquatic degradation	• FPB-acid Content increased continuously up to 70% of applied radioactivity at day 21 • 4'OH-FPB-acid (4-fluoro-3-(4-hydroxyphenoxy)-benzoic acid, FCR 3145), • COOH-cyfluthrin (α-[[[3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropyl]carbonyl]oxy]-4-fluoro-3-phenoxybenzeneacetic acid, FCR 2728). Up to 2.5% of applied radioactivity in total
Water sediment	• FPB-acid (4-fluoro-3-phenoxybenzoic acid, FCR 3191, CAS-no.: 77279-89-1) maximum of 44.5% of applied radioactivity • FPB-ald maximum of 15.7% of applied radioactivity • DCVA

	maximum of 47.6% of applied radioactivity • COOH-cyfluthrin • $CONH_2$-cyfluthrin (cyclopropanecarboxylic acid, 3-(2,2-dichloroethenyl)-2,2-dimethyl-, 2-amino-1-(4-fluoro-3-phenoxyphenyl)-2-oxoethyl ester, FCR 2947) • 4'OH-FPB-acid <10% of applied radioactivity in total

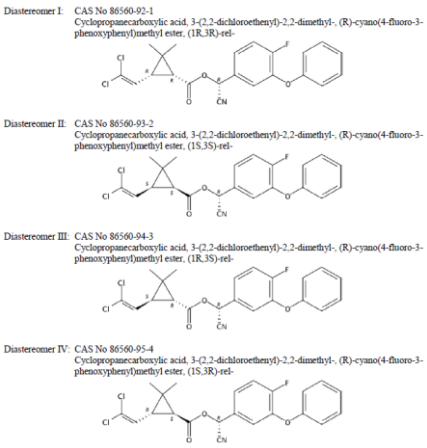
The DS concluded that as cyfluthrin does not ultimately degrade to greater than 70% in aquatic systems and undergoes to primary degradation to classifiable products, cyfluthrin does not fulfil the criteria to be considered as rapidly degradable in the aquatic environment. This is based on data for cyfluthrin.

<u>Justification of read-across of data to cyfluthrin from beta-cyfluthrin for bioaccumulation and aquatic toxicity</u>

Cyfluthrin and beta-cyfluthrin are mixtures of eight isomers, four of the isomers are considered active. The proportion of diastereoisomer pairs and their structures in cyfluthrin and beta-cyfluthrin is 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**.



Cyfluthrin consists of approximately 40% beta-cyfluthrin, therefore representing a major constituent. Cyfluthrin and beta-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) and plant protection evaluation (beta-cyfluthrin) that both substances share a similar toxicological profile.

There are indications from scientific literature that diastereomers I and III could be regarded as around one order of magnitude less active than isomers II and IV (Leicht, 1996). If only diastereomers II and IV would be biologically active, cyfluthrin would be approximately 2.4 times less toxic as beta-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 levels of relevance of data for both substances can be concluded and effect studies with beta-cyfluthrin are considered for the hazard assessment of cyfluthrin.

Bioaccumulation

Cyfluthrin consists of four diastereoisomers I-IV with log K_{OW} values ranging from 5.91 for diastereoisomer IV to 6.04 for diastereoisomer III. 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 ranged between 27164 L/kg_{wet} in fish, for diastereoisomer III, and 21062 L/kg_{wet} in fish, for diastereoisomer IV.

From a study similar to OECD TG 305 of reduced reliability with Bluegill sunfish, a BCF of 854 L/kg_{wet} fish for cyfluthrin has been derived, which represents the highest value from the study, despite no stable steady state plateau being reached within the uptake period of 28 days.

Another bioaccumulation study following a flow-through test design according to OECD TG 305 with *Lepomis macrochirus* and radio-labelled beta-cyfluthrin has been provided. A reliable BCF_k of 1822 L/kg_{wet} fish was derived. The DS adds that in the case of bioaccumulation it is appropriate to conclude that data for beta-cyfluthrin is relevant for cyfluthrin, especially because the study available for cyfluthrin exhibits significant shortcomings (see section on justification of read-across of data to cyfluthrin from beta-cyfluthrin).

The DS considers cyfluthrin as having a high potential for bioaccumulation based on reliable Log K_{ow}s > 4 for diastereomers of cyfluthrin and a BCF_k > 500 for beta-cyfluthrin.

Aquatic toxicity

Summary of relevant information on aquatic toxicity of cyfluthrin

Test	Test species	Result **µg/L**	Reference
Fish			
cyfluthrin (purity 97.6%) US EPA FIFRA G. 72-1 equivalent to OECD TG 203 flow-through, 96 h	*Oncorhynchus mykiss*	LC_{50} = 0.302 based on mean measured concentrations	Anonymous (1994) Report 106652 A 7.4.1.1/01
cyfluthrin (purity 97.6%) FIFRA G. 72-1 equivalent to OECD TG 203 flow-through, 96 h	*Lepomis macrochirus*	LC_{50} = 0.998 based on mean measured concentrations	Anonymous (1994) Report 106774 A 7.4.1.1/02
cyfluthrin (purity 96.6%) OECD TG 203	*Cyprinus carpio*	LC_{50} = 5.57 based on mean	Anonymous (2004)

flow-through, 96 h		measured concentrations	Report EBBDU004 A 7.4.1.1/03
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 (1985) Report 683 A 7.4.3.2/01
^{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 (1990) Report 100097
Invertebrates			
cyfluthrin (purity 98.6%) EPA G. 72-2 (1982) equivalent to OECD TG 202 flow-through, 48 h	*Daphnia magna*	LC_{50} = 0.16 based on mean measured concentrations	Burgess, D. (1990) A 7.4.1.2/01
cyfluthrin (purity 97%) ASTM, 1980 flow-through, 96 h	*Procambarus clarkii*	LC_{50} = 0.062 based on mean measured concentrations	Suprenant, D.C. (1990) A 7.4.1.2/02
cyfluthrin (purity 95.8%) OCSPP draft 850.1020 flow-through, 96 h	*Hyalella azteca*	**LC_{50} = 0.00055** based on mean measured concentrations	Bradley, M.J. (2013) A7.4.1.2/05
cyfluthrin (purity 94.7%) ASTM Draft No. 3 (1981) equivalent to OECD TG 211 flow-through, 21 d	*Daphnia magna*	NOEC = 0.02 (reproduction and adult length) based on mean measured concentrations	Forbis, A. D. (1984) A 7.4.3.4
beta-cyfluthrin (purity 99.2%) OCSPP draft 850.1350 flow-through, 28 d	*Americamysis bahia*	**NOEC = 0.00041** based on mean measured concentrations	Schwader, A.L. (2013) A7.4.3.4/02
Algae/Other aquatic organisms			
cyfluthrin (purity 96.6%) Draft Proposal for Updating OECD Guideline 201 (2004), JMAFF guideline (2000) static, 72 h	*Pseudokirchneriella subcapitata*	NOE_rC = 4.45 mg/L E_rC_{50} > 8.05 based on initial mean measured concentrations	Dorgerloh, M. (2004) A 7.4.1.3
cyfluthrin (purity 99%) EPA - Springborn Smithers Protocol No.: 051704 spiked sediment with renewal of overlying water per day, 10 d	*Chironomus tentans*	LC_{50} = 280 µg/kg dw based on mean measured sediment concentrations	Putt, A (2005) A7.4.3.5.1/01
cyfluthrin (purity 95.8%) EPA 100.5, 850.SUP and SS-1069 spiked sediment with renewal of overlying water, 63 d	*Chironomus dilutus*	Emergence: 6.2 µg/kg dw based on mean measured sediment concentrations	Picard, C.R. (2013a) A 7.4.3.5.1/02
cyfluthrin (purity 95.8%) EPA 100.5, 850.SUP and SS-1069 spiked sediment with renewal of overlying	*Hyalella azteca*	NOEC = 20 µg/kg dw based on mean measured sediment concentrations	Picard C.R. (2013b) A 7.4.3.5.1/03

water, 42 d			
cyfluthrin (purity 93.3%) EPA Guideline series 850 spiked sediment with renewal of overlying water, 28 d	*Leptocheirus plumulosus*	NOEC = 13 µg/kg dw based on mean measured sediment concentrations	Putt, A.E. (2005a) A 7.4.3.5.1/04

Acute Aquatic Toxicity

Acute toxicity of cyfluthrin to fish was investigated in studies that were considered reliable and equivalent to OECD TG 203. Three different fish species (Rainbow trout, Bluegill sunfish and Common carp) were exposed under flow-through conditions for 96 h. The LC_{50} values ranged from 0.000302 mg/L to 5.57 µg/L. The lowest no observed effect level out of the three acute fish toxicity tests was 0.105 µg/L. The results are based on mean measured concentrations.

The acute toxicity of cyfluthrin to invertebrates was investigated in flow-through tests with Daphnia, crayfish, and *Hyalella azteca* according to ASTM and EPA methods, which can be considered as equivalent to the corresponding OECD test guidelines. The tests results were all based on mean measured concentrations and considered reliable. The most sensitive species was *Hyalella azteca*, the 96 h study was performed without sediment with nominal concentrations of 0.20, 0.40, 0.80, 1.6 and 3.2 ng/L (measured concentrations: 0.17, 0.32, 0.60, 1.2 and 2.6 ng/L). Based on mortality, a **LC_{50} = 0.00055 µg/L** was derived.

The acute toxicity of cyfluthrin to midge in a water-sediment system was also determined, resulting in an LC_{50} of 280 µg/kg dw based on mean measured sediment concentrations and survival as the most sensitive parameter.

Chronic Aquatic Toxicity

The chronic toxicity of cyfluthrin to two fish species (Rainbow trout and Fathead minnow) was investigated according to a flow-through test procedures, 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.

The presented acceptable Daphnia chronic study was conducted in accordance with an ASTM method in principle comparable to OECD TG 211. The 21-d NOEC value for reproduction and adult length was 0.02 µg/L based on mean measured concentration and for adult survival 0.041 µg/L, 100% mortality occurred at the highest mean measured concentration of 0.22 µg/L.

Considering the acute effect data for invertebrates, *Daphnia magna* has been shown to be two orders of magnitude less sensitive than the most sensitive species tested, *H. azteca*. Long-term data for cyfluthrin is only available for *D. magna*, which would not cover the differences in species sensitivity observed in the acute dataset. However, for beta-cyfluthrin a relevant additional chronic study with the marine mysid *A. bahia* is available and has been considered for hazard assessment (see above section on justification of read-across of data to cyfluthrin from beta-cyfluthrin).

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** for beta-cyfluthrin (mean measured) was derived.

Three tests presented that studied the long-term toxicity of cyfluthrin on benthic organisms *C. dilutus*, *H. azteca* and *L. plumulosus* resulted in a 63-d NOEC of 6.2 µg/kg dw (emergence), 42-d

NOEC of 20 µg/kg dw (survival, growth and emergence) and in a 28-d NOEC of 13 µg/kg dw (survival and growth), respectively, based on mean measured sediment concentrations.

Toxicity in Algae

A study with *P. subcapitata* in accordance with the draft proposal for updating OECD TG 201 has been presented. There are discrepancies between the nominal and the measured concentrations of cyfluthrin in the test medium and the algae growth in the control and solvent control cultures do not follow a monotonal exponential growth. In addition, the effect value exceeds the water solubility of cyfluthrin by several orders of magnitude. However, the test is considered acceptable for hazard assessment, especially as algae are not the critical species for the aquatic hazard assessment.

Comments received during public consultation

Two MSCAs supported the proposed environmental classification of Aquatic Acute 1, H400 (M = 1000000) and Aquatic Chronic 1, (M = 100000) based on the available data for the most sensitive species (Invertebrates: *H. Azteca*; 96-h LC_{50}= 0.55 ng/L and *A. bahia* 28-d NOEC=0.41 ng/L), respectively.

One MSCA commented that the study on which the acute classification is based (Bradley, 2013) can be considered valid and reliable for hazard classification. Regarding using the available chronic endpoint 28-d NOEC of 0.00000041 mg/L (mm) for *A. bahia*, it was noted that beta-cyfluthrin is anticipated to be more toxic than cyfluthrin and the surrogate approach using the *H. Azteca* acute endpoint for cyfluthrin chronic classification results in an M-factor of 1000000. It was further noted that *H. Azteca* appears to be more sensitive to the active isomers in cyfluthrin and beta-cyfluthrin on the basis of a less sensitive acute 96-h LC_{50} of 0.0000022 mg/L (mm) for *A. bahia* using beta-cyfluthrin.

The same MSCA also agreed that algae are not likely to be the most sensitive species for hazard assessment but did not agree that the presented acute toxicity study with *P. subcapitata* is suitable for definitive hazard classification as the study controls were not valid.

Assessment and comparison with the classification criteria

Taking into account the argumentation for read-across of aquatic toxicity data from beta-cyfluthrin to cyfluthrin based on cyfluthrin consisting of approximately 40% beta-cyfluthrin (II + IV) and sharing the same chemical structure as well as the corresponding diastereomers, RAC agrees with the DS that read-across from beta-cyfluthrin to cyfluthrin can be made for bioaccumulation and aquatic toxicity in algae.

RAC agrees with the DS submitter that based on data for cyfluthrin, there is no evidence that cyfluthrin degrades to a degree greater 70% over 28 days. Cyfluthrin is subject to rapid primary degradation (DT_{50} < 16 days) via photodegradation and in water/sediment systems. However, as some of the degradation metabolites appear to meet the criteria for hazardous to the aquatic environment under CLP, cyfluthrin cannot be considered as rapidly degradable in the aquatic environment. Therefore, RAC agrees with the DS to consider 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 diastereomers of cyfluthrin. Consequently, RAC agrees with the DS that cyfluthrin is bioaccumulative in the aquatic environment.

There are reliable experimental data on acute toxicity on fish and invertebrates available for cyfluthrin, the lowest value being a 96-h LC_{50} for *H. azteca* of 0.00055 µg/L.

There is reliable experimental data on chronic toxicity for fish and invertebrates for cyfluthrin. However, RAC does not consider the algal data for cyfluthrin reliable for classification purposes due to the previously mentioned issues (regarding monitoring data and algal control growth). RAC considers the read-across acceptable and uses aquatic toxicity data for algae using beta-cyfluthrin to complete the data set, even though this makes no difference to the classification outcome. In the read-across algae study, beta-cyfluthrin was tested at one concentration of 0.01 mg as/L as higher test concentrations could not be examined due to low water solubility. No effects were seen at this concentration (NOEC ≥ 0.01 mg/L for biomass and the growth rate).

Having accepted the read-across from beta-cyfluthrin, the lowest available chronic toxicity value is a 28-d NOEC for *A. bahia* 0.00041 µg/L based on a study with beta-cyfluthrin. However, it can be expected that, based on the content of biological active isomers, beta-cyfluthrin is possibly up to 2.4 or 1.3 times more toxic than cyfluthrin. Therefore, classifying cyfluthrin using beta-cyfluthrin data as proposed by the DS may underestimate the chronic effects of cyfluthrin on the most sensitive species (*H. azteca*). As there is no chronic data for the most sensitive species under acute testing for cyfluthrin, RAC considers it appropriate to use acute data with cyfluthrin under the surrogate approach to derive a chronic classification for cyfluthrin.

The chronic classification derived using the 28-d NOEC for *A. bahia* of 0.00000041 mg/L using beta-cyfluthrin, results in a classification of Aquatic Chronic 1, M=100000, for a not rapidly degradable substance. Although RAC considers the read-across acceptable, the acute 96-h LC_{50} for *H. azteca* of 0.00000055 mg/L with cyfluthrin results in a more stringent classification of Aquatic Chronic 1, M = 1000000, following CLP table 4.1.0(b)(iii) and 4.1.3.

Data that RAC considers for comparison with the CLP criteria are summarised in the table below.

Table: *Summary of data for classification of cyfluthrin*

Results	**Test substance**	**Remarks**
Fish		
96-h LC_{50} = 0.000302 mg/L *Onchorhynchus mykiss*	cyfluthrin	
58-d NOEC = 0.000010 mg/L *Oncorhynchus mykiss*	cyfluthrin	growth
Invertebrates		
96-h LC_{50} = 0.00000055 mg/L *Hyalella azteca*	cyfluthrin	
21-d NOEC = 0.000020 mg/L *Daphnia magna*	cyfluthrin	
Algae/Aquatic plants		
E_rC_{50} = > 10 µg/L NOE_rC = 10 µg/L *Scenedesmus subspicatus*	beta-cyfluthrin	read-across from beta-cyfluthrin

Note – following use of the surrogate approach, chronic data for beta-cyfluthrin is no longer used.

In conclusion, RAC agrees with the DS that based on an LC_{50} of 0.00000055 mg/L for *H. azteca*, cyfluthrin warrants classification as **Aquatic Acute 1 (H400), M=1000000**.

However, for chronic classification RAC disagrees with the DS and proposes to classify cyfluthrin as **Aquatic Chronic 1 (H410), M=1000000** based on the surrogate approach using the LC_{50} of 0.00000055 mg/L for *H. Azteca*, as no chronic data is available for this species.

RAC notes that if long-term data on *H. Azteca* becomes available, the classification could be reconsidered.

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ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).