Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

esfenvalerate (ISO); (*S*)-α-cyano-3-phenoxybenzyl-(*S*)-2-(4-chlorophenyl)-3-methylbutyrate

EC Number: -
CAS Number: 66230-04-4

CLH-O-0000006715-69-01/F

Adopted

20 September 2019

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CLH-O-0000006715-69-01/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: **esfenvalerate (ISO); (*S*)-α-cyano-3-phenoxybenzyl-(*S*)-2-(4-chlorophenyl)-3-methylbutyrate**

EC Number: **-**

CAS Number: **66230-04-4**

The proposal was submitted by the **United Kingdom** and received by RAC on **13 November 2018.**

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

PROCESS FOR ADOPTION OF THE OPINION

The **United Kingdom** 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 **17 December 2018**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **1 March 2019**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Anna Biró**

Co-Rapporteur, appointed by RAC: **Žilvinas Užomeckas**

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 **20 September 2019** 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	608-058-00-4	esfenvalerate (ISO); (*S*)-α-cyano-3-phenoxybenzyl-(*S*)-2-(4-chlorophenyl)-3-methylbutyrate	-	66230-04-4	Acute Tox. 3* Acute Tox. 3* Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H301 H331 H317 H400 H410	GHS06 GHS09 Dgr	H301 H331 H317 H410		M=10000	
Dossier submitters proposal	608-058-00-4	esfenvalerate (ISO); (*S*)-α-cyano-3-phenoxybenzyl-(*S*)-2-(4-chlorophenyl)-3-methylbutyrate	-	66230-04-4	**Retain** Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1 **Add** STOT RE 2 **Modify** Acute Tox. 3 Acute Tox. 2	**Retain** H317 H400 H410 **Add** H373 **Modify** H301 H330	**Retain** GHS06 GHS09 Dgr **Add** GHS08	**Retain** H317 H410 **Add** H373 **Modify** H301 H330		**Retain** M=10000 **Add** oral; ATE = 88.5 mg/kg bw inhal; ATE = 0.48 mg/L M=10000	
RAC opinion	608-058-00-4	esfenvalerate (ISO); (*S*)-α-cyano-3-phenoxybenzyl-(*S*)-2-(4-chlorophenyl)-3-methylbutyrate	-	66230-04-4	**Retain** Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1 **Add** STOT SE 1 STOT RE 2 **Modify** Acute Tox. 3 Acute Tox. 3	**Retain** H317 H400 H410 **Add** H370 (nervous system) H373 **Modify** H301 H331	**Retain** GHS06 GHS09 Dgr **Add** GHS08	**Retain** H317 H410 **Add** H370 (nervous system) H373 **Modify** H301 H331		**Retain** M=10000 **Add** oral; ATE = 88.5 mg/kg bw inhal; ATE = 0.53 mg/L (dusts or mists) M=10000	
Resulting Annex VI entry if agreed by COM	608-058-00-4	esfenvalerate (ISO); (*S*)-α-cyano-3-phenoxybenzyl-(*S*)-2-(4-chlorophenyl)-3-methylbutyrate	-	66230-04-4	Acute Tox. 3 Acute Tox. 3 STOT SE 1 STOT RE 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H331 H301 H370 (nervous system) H373 H317 H400 H410	GHS06 GHS08 GHS09 Dgr	H331 H301 H370 (nervous system) H373 H317 H410		oral; ATE = 88.5 mg/kg bw inhal; ATE = 0.53 mg/L (dusts or mists) M=10000 M=10000	

GROUNDS FOR ADOPTION OF THE OPINION

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute oral toxicity

The DS summarised four studies: two acute oral toxicity studies (rat, mouse) and two acute oral neurotoxicity studies (rat) in the CLH report.

In the **first acute oral toxicity study** (Anonymous, 1985d), carried out according to the OECD TG 401, Sprague-Dawley rats (10 animals/sex/group) were given doses of 0, 5, 10, 20, 40, 55, 75, 100, 130 or 180 mg/kg bw esfenvalerate, technical grade (87.2%), in corn oil by gavage. Mortality was observed from 55 mg/kg bw with 100% mortality at the highest dose. Gastric haemorrhage was noted in animals that died during the study. Signs of toxicity were observed from 10 mg/kg and were typical of the transient clinical signs associated with pyrethroid toxicity (muscular fibrillation, tremors, decrease of spontaneous activity, ataxia, limb paralysis, irregular respiration, dyspnoea, salivation, hyper-excitability and choreothetotic syndrome). The signs of toxicity resolved in all animals within three days. There were no treatment-related gross pathological findings in animals surviving to scheduled termination after the 14-day observation period. The acute oral LD_{50} value for esfenvalerate in both male and female rats was 88.5 mg/kg bw.

In the **second acute oral toxicity study** (Anonymous, 1986a), also done according to the OECD TG 401, ICR mice (10 animals/sex/group) were given oral gavage doses of esfenvalerate in aqueous 0.5% methyl cellulose solution at 0, 5, 15, 50, 70, 100, 140, 200, 280 or 400 mg/kg bw. The test material was technical grade (87.2%) esfenvalerate. There was 10, 30, 60 and 90% mortality with female mice dosed 140, 200, 280 and 400 mg/kg bw, respectively and 20, 30 and 100% mortality in male mice dosed 200, 280 and 400 mg/kg bw, respectively. Gastric haemorrhage was noted in animals that died during the study. Transient clinical signs of toxicity similar to those seen in the rat were observed from 15 mg/kg bw and had resolved within 2 days after dosing. There were no treatment-related gross pathological findings in animals surviving to scheduled termination after the 14-day observation period. The acute oral LD_{50} values for esfenvalerate were 320 mg/kg bw (male) and 250 mg/kg bw (female).

Two acute oral neurotoxicity studies done according to OECD TG 424 in Sprague-Dawley rats are also deemed as relevant for the classification proposal by the DS. (These studies are elaborated in the section for STOT SE). In one study (Anonymous, 2000a), no mortalities were observed up to the top dose of 80 mg/kg bw. In the other study (Anonymous, 1985e), 2/8 males and 1/8 female died at the top dose of 90 mg/kg/bw, thus the estimated LD_{50} for this study is >90 mg/kg bw.

Conclusion

The DS concluded that the rat is the most sensitive species, and using the lowest LD_{50} value of 88.5 mg/kg bw for both males and females, proposed to classify esfenvalerate as Acute Tox. 3; H301: Toxic if swallowed, with an ATE of 88.5 mg/kg bw.

Acute inhalation toxicity

One guideline (OECD TG 403) study (Anonymous, 1985f) was discussed in the CLH report. Sprague-Dawley rats (10 animals/sex/group) were exposed whole-body to atmospheric concentrations of 0, 2.40, 13.8, 205, 395, 550 and 1130 mg/m^3 of esfenvalerate technical grade (87.2%) diluted in corn oil, for 4 hours. The MMAD of the particles ranged from 0.94 to 1.07 µm. The control animals were exposed to compressed air only and another group exposed to corn oil spray alone. There was 10, 90 and 100% mortality in male rats exposed to concentrations of 395, 550 and 1130 mg/m^3 of esfenvalerate, respectively, while female rats showed 20, 20 and 100% mortality at the same concentrations of test material. Deaths occurred within 2 hours after termination of exposure. Autolysis of the intestinal tract was observed in animals that died during the study. Signs of toxicity at and above 205 mg/m^3 included hyperpnoea, dyspnoea, nasal discharge, urinary incontinence, hypersensitivity to sound, muscular fibrillation, abnormal gait, decrease of spontaneous activity, ataxia, lacrimation and salivation. These signs all resolved within 2 days of exposure. There were further signs of neurological effects consistent with pyrethroid toxicity at ≥395 mg/m^3 (choreoathetotic movement, tremors and aggressive sparring). All signs of toxicity had completely resolved within 5 days after exposure. There were no treatment related gross or histopathological findings in the respiratory tract of animals surviving to termination 14 days after exposure. The acute inhalation 4-hr LC_{50} of esfenvalerate was 0.48 mg/L in male, and 0.57 mg/L in female rats.

Conclusion

The DS concluded that the lowest LC_{50} of 0.48 mg/L in male rat is the relevant value, and proposed to classify esfenvalerate as Acute Tox. 2; H330: Fatal if inhaled, with an ATE of 0.48 mg/L for dusts/mists.

Comments received during public consultation

Three member state competent authorities supported the classification proposed by the DS.

Assessment and comparison with the classification criteria

Esfenvalerate belongs to the family of synthetic pyrethroid insecticides which act on the sodium channel in the nerve membranes (sodium channel modulators), causing a prolongation of the transient increase in sodium permeability of the nerve membranes. This results in continual nerve impulse transmission leading to tremors and death. Sodium channels are also found in mammals and therefore humans are also potential targets for the neurotoxicity of pyrethroids. Studies in animals confirm that acute pyrethroid intoxication is associated with altered nerve function, principally involving the brain, spinal cord, and elements of the peripheral nervous system, predominantly via interaction with the voltage-gated membrane sodium channel and to some extent the chloride and calcium channels. The transient neurological effects tend to correlate with peak blood concentrations and usually dissipate within several hours to a day or so after a single gavage dose as a result of metabolism and excretion.

Oral route

In the acute oral toxicity studies, an acute oral LD_{50} value of 88.5 mg/kg bw was reported for male/female rats, and acute oral LD_{50} values of 320 and 250 mg/kg bw were reported for male and female mice, respectively. In one acute oral neurotoxicity study the estimated LD_{50} is >90 mg/kg bw, while no mortalities were observed up to the top dose of 80 mg/kg bw in the second study.

The lowest oral LD_{50} value was found in rats, with a value of 88.5 mg/kg bw for both males and females. The criteria for classification with acute oral toxicity category 3 are $50 < LD_{50} \leq 300$. Therefore, RAC supports the DS's proposal to classify esfenvalerate as **Acute Tox. 3; H301: Toxic if swallowed, and proposes an oral ATE of 88.5 mg/kg bw.**

Inhalation route

In a guideline acute inhalation toxicity study, the acute inhalation 4-hr LC_{50} of esfenvalerate was 0.48 mg/L in male, and 0.57 mg/L in female rats. It has to be noted that whole-body exposure to corn oil spray is likely to lead to high oral exposure via grooming, which is corroborated by the autolysis of the intestinal tract of the animals that died. Thus, the observed LC_{50} values probably reflect exposure both via inhalation and the oral route. As the values overestimate inhalation toxicity, it is proposed to use the mean of the male and female values for calculating the LC_{50} which leads to 0.53 mg/L.

The criteria for classification with acute inhalation toxicity category 3 (inhalation, dust/mist) are $0.5 < LC_{50} \leq 1.0$ mg/L. Therefore, RAC concluded that esfenvalerate warrants classification as **Acute Tox. 3; H331: Toxic if inhaled, with an ATE of 0.53 mg/L (dusts/mists).**

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

Summary of the Dossier Submitter's proposal

For **specific target organ toxicity – single exposure**, the DS summarised two acute oral neurotoxicity studies (rat), two acute oral toxicity studies (rat, mouse), two acute dermal toxicity studies (rat, rabbit) and an acute inhalation toxicity study (rat).

Oral studies

In the **first acute oral neurotoxicity study** (Anonymous, 2000a), done according to the OECD TG 424, Sprague-Dawley rats (10 animals/sex/group) were given single doses of 0, 1.75, 1.90, 20 or 80 mg/kg bw esfenvalerate in corn oil by gavage. The test material was technical grade esfenvalerate (purity not reported). There was no mortality reported in the study. At 1.9 mg/kg bw tremors occurred in one female, at 20 mg/kg bw stereotypical grooming and tremors were seen in occasional animals. At 80 mg/kg bw a number of changes were observed, namely soiled fur, salivation, tremors, un-coordination, stereotypical grooming, abnormal gait, diarrhoea, paw shaking in both genders, slow righting reflex and increased reaction to touch or tail pinch, reduced motor activity, reduced forelimb grip strength and hind limb foot splay, reduced body weight gain and reduced food consumption. All signs were resolved by 4 days after dosing. No microscopic neurological lesions were observed at any dose level.

In the **second acute oral neurotoxicity study** (Anonymous (1985e), done according to the OECD TG 424, Sprague-Dawley rats (8 animals/sex/group) were given single doses of 0, 5, 20 or 90 mg/kg bw esfenvalerate in corn oil by gavage. The test material was technical grade (87.2%) esfenvalerate. Three animals receiving 90 mg/kg bw esfenvalerate were found dead within 24 hours of dosing. Clinical signs of toxicity were seen from 2 hours after dosing, such as muscular fibrillation, hunched posture and ataxia in the 20 and 90 mg/kg bw dose groups. Tremor and limb paralysis were also observed in some animals from the high dose (90 mg/kg bw) group. No treatment related clinical signs of toxicity were observed at doses of up to 5 mg/kg bw esfenvalerate. All clinical signs of toxicity had resolved within 2 days of dosing. Slight to minimal axonal degeneration and/or demyelination with Schwann cell proliferation in peripheral nerves

were noted at the highest dose. No pathological lesions were observed at non-lethal doses where neurological clinical signs were present.

In the **first acute oral toxicity study** (elaborated in the acute toxicity section, Anonymous, 1985d), mortality occurred from 55 mg/kg bw. At 10 mg/kg bw transient muscular fibrillation and decrease of spontaneous activity, at 40 mg/kg transient muscular fibrillation, occasional signs of tremor, limb paralysis and ataxia were observed. At 55 mg/kg bw and above, muscular fibrillation, tremors, decrease of spontaneous activity, ataxia, limb paralysis, irregular respiration, dyspnoea, salivation, hyper-excitability and choreoathetotic syndrome were noted. These signs gradually developed one hour after dosing, however, they had disappeared in all animals within 3 days and generally within 2 days.

In the second **acute oral toxicity study** (elaborated in the acute toxicity section, Anonymous, 1986a), mortality was noted from 140 mg/kg bw in female mice, and 200 mg/kg bw in male mice. At 15 mg/kg bw transient muscular fibrillation and decrease of spontaneous activity, at 70 mg/kg bw and 100 mg/kg bw transient muscular fibrillation, occasional signs of tremor, limb paralysis and ataxia were noted. At 140 mg/kg bw and above transient signs of toxicity included muscular fibrillation, tremors, decrease of spontaneous activity, ataxia, limb paralysis, irregular respiration and salivation. These signs gradually developed 10 minutes after dosing; however, they disappeared in all surviving animals within 2 days.

Dermal studies

In the **first acute dermal toxicity study** (Anonymous, 1985m), done according to the OECD TG 402, Sprague-Dawley rats (10 animals/sex/group) were dosed at 0, 500, 1000, 2000, 3200 and 5000 mg/kg bw in corn oil. The test material was technical grade (87.2%) esfenvalerate. No mortality occurred. At 1000 mg/kg bw transient muscular fibrillation, at 2000 mg/kg bw and above transient muscular fibrillation, decrease of spontaneous activity, ataxia, irregular respiration and urinary incontinence were noted. These signs of toxicity developed 2 - 4 hours after application but had disappeared within 8 days. The NOAEL was 500 mg/kg bw.

In the **second acute dermal toxicity study** (Anonymous, 1985j), done according to the OECD TG 402, New Zealand White rabbits (5 animals/sex) were dosed at a single dose level of 2000 mg/kg bw (undiluted). The control group consisted of 5 females. The test material was technical grade (87.2%) esfenvalerate. Signs of toxicity included decreased activity, ataxia, body tremors, constricted pupils, decreased defecation and urination, diarrhoea, emaciation, muscle tremors, poor hind limb co-ordination and small faeces. There was no treatment related mortality according to the study report. One death (10%) occurred in the treated group and one death (20%) occurred in the vehicle (water) control group (the latter leading the active substance Renewal Review and the DS to question the reliability of the study). The post mortem signs in both animals were similar: diarrhoea, emaciation, nasal discharge, salivation, gastrointestinal tract distended with gas and discoloration of the intestinal tract.

Inhalation study

In the **acute inhalation toxicity study** (elaborated in the acute toxicity section, Anonymous, 1985f), mortalities occurred from 395 mg/m^3. At 13.8 mg/m^3 some rats showed irregular respiration, but this disappeared within 1 hour after termination of exposure. At 205 mg/m^3 and above signs of toxicity included hyperpnoea, dyspnoea, nasal discharge, urinary incontinence, hypersensitivity to sound, muscular fibrillation, abnormal gait, decrease of spontaneous activity, ataxia, lachrymation and salivation, however, all these signs disappeared within 2 days of the exposure. Choreoathetotic movement, tremors and aggressive sparring were observed in rats exposed to concentrations of ≥395 mg/m^3. All signs of toxicity had completely cleared within 5 days after exposure.

Conclusion

As to the classification of esfenvalerate, the DS argued that STOT SE 3 was not appropriate as the profile of effects seen with esfenvalerate was considered not to be indicative of narcosis, and based on the available data respiratory tract irritation was not appropriate either.

Concerning STOT SE 1 or STOT SE 2, the DS argued that in acute oral toxicity and acute oral neurotoxicity studies in rats and mice, as well as in an acute inhalation study in rats, dose-dependent significant and severe signs of toxicity (neurological effects, death) were observed. The neurotoxicity consistent with the well-known mechanism of action of pyrethroids allowed the DS to propose that the lethality was due to neurotoxicity. As Acute Tox. 3 (H301) and Acute Tox. 2 (H330) was proposed by the DS, classification for STOT SE was not considered appropriate, as it would result in a double classification. Also, in an acute dermal toxicity study in the rat, relatively minor transient neurological signs were not considered to be indicative of significant or severe toxicity in the context of STOT SE Category 1 (C ≤ 1000 mg/kg bw) or Category 2 (2000 ≥ C > 1000 mg/kg bw). Transient neurological signs at the single dose level of 2000 mg/kg bw were found in a rabbit acute dermal toxicity study in the presence of other significant general toxicity, but as the reliability of this study was questioned by the DS, the results were not deemed to support classification in STOT SE 1 or 2. Overall, the DS proposed not to classify esfenvalerate for STOT SE.

Comments received during public consultation

Two MSCAs supported the proposal of the DS not to classify esfenvalerate for STOT SE.

Assessment and comparison with the classification criteria

In the available acute toxicity and neurotoxicity studies, esfenvalerate induced neurotoxicity following acute oral, inhalation and dermal exposure.

In acute oral neurotoxicity and acute oral toxicity studies in rats and mice, clinical signs indicative of neurotoxicity appeared 1-2 hours (rats) or 10 minutes (mice) post dosing. In surviving animals these signs disappeared within 2-3 days. The signs of neurotoxicity were muscular fibrillation, hunched posture, ataxia, tremors, un-coordination, stereotypical grooming, abnormal gait, reduced motor activity, reduced forelimb grip strength and hind limb foot splay. The severity of the neurological effects increased in a dose-dependent manner, and were observed at doses not resulting in mortality.

In one acute oral neurotoxicity study (Anonymous, 2000a), there was no mortality, but signs of neurotoxicity started at 1.9 mg/kg bw (tremors in one female). At 20 mg/kg bw stereotypical grooming and tremors were seen in occasional animals and at 80 mg/kg (top dose) signs included tremors, un-coordination, stereotypical grooming, abnormal gait, paw shaking, slow righting reflex, increased reaction to touch or tail pinch, reduced motor activity, reduced forelimb grip strength and hind limb foot splay.

In the other oral neurotoxicity study (Anonymous (1985e) mortality occurred from 90 mg/kg bw, but muscular fibrillation, hunched posture and ataxia were observed already at a dose of 20 mg/kg bw, well below the dose causing mortality.

In the acute oral toxicity study in rats (Anonymous 1985d), mortality occurred from 55 mg/kg bw, but signs of neurotoxicity started at 10 mg/kg bw (muscular fibrillation and decreased activity), while at 40 mg/kg bw muscular fibrillation, occasional signs of tremor, limb paralysis and ataxia were observed.

In the acute oral toxicity study in mice (Anonymous, 1986a) signs of neurotoxicity were seen from 15 mg/kg bw (muscular fibrillation and decrease of spontaneous activity). At 70 mg/kg and 100 mg/kg transient muscular fibrillation, occasional signs of tremor, limb paralysis and ataxia were noted. At 140 mg/kg and above muscular fibrillation, tremors, decrease of spontaneous activity, ataxia, limb paralysis, irregular respiration and salivation were observed. Mortality was noted from 140 mg/kg in female mice, and 200 mg/kg in male mice, therefore the neurotoxic effects at 15 and 70 mg/kg bw were seen well below doses causing mortality.

In the acute dermal studies in rats, signs of neurotoxicity started at 1000 mg/kg bw (muscular fibrillation), while muscular fibrillation, decrease of spontaneous activity, ataxia, irregular respiration and urinary incontinence developed at a dose of 2000 mg/kg bw. No mortality occurred up to the top dose of 5000 mg/kg bw. In the dermal study in rabbits, only one dose was investigated: at 2000 mg/kg bw the neurotoxic signs were decreased activity, ataxia, body tremors, constricted pupils, muscle tremors, and poor hind limb co-ordination. There was no mortality in this dermal study either.

In the inhalation study (Anonymous 1985f) mortality occurred from 395 mg/m^3. At 13.8 mg/m^3 some rats showed irregular respiration, and at 205 mg/m^3 and above signs of toxicity included hyperpnoea, dyspnoea, nasal discharge, urinary incontinence, hypersensitivity to sound, muscular fibrillation, abnormal gait, decrease of spontaneous activity, ataxia, lachrymation and salivation.

The effects are related to the neurotoxic mode of action of esfenvalerate, a synthetic pyrethroid insecticide that acts on the sodium channel in the nerve membranes. Sodium channels are also found in mammals and therefore humans are also potential targets for the neurotoxicity of pyrethroids. Indeed, pyrethroid-induced paresthesia is frequently seen after dermal exposure to pyrethroids. Affected individuals experience a sensation of burning, tingling, itching, or numbness, most commonly in the face.

Conclusion

Neurotoxicity was consistently observed across all acute oral, dermal and inhalation studies, at both lethal and non-lethal doses. The substance is already proposed to be classified for lethality, but the fact that effects are also seen at non-lethal doses makes it necessary to consider if additional classification for STOT SE is warranted. As the overall profile of toxic signs is not typical of narcosis or respiratory tract irritation, classification with STOT SE 3 is not appropriate. The non-lethal doses at which the neurotoxic effects are observed fall within the guidance values for STOT SE 1 for the oral (C ≤ 300 mg/kg bw) and inhalation (≤ 1 mg/L) routes, and within the guidance values for STOT SE 2 C ≤ 2000 mg/kg bw) for the dermal route. The sublethal dose levels with neurotoxic findings were, with the exception of the inhalation route, more than a factor of 2 lower than the lethal dose levels. The severity and incidence of the neurotoxic effects was dose dependent.

Given the consistent pattern of effects, across all routes of exposure, supported by the fact that esfenvalerate belongs to the group of pyrethroids, which are known to induce neurotoxic effects, RAC supports **classification as STOT SE 1; H370 (nervous system)** without specifying the route of exposure.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

Three skin sensitisation studies were discussed in the CLH report, 1 guinea pig maximisation test (GPMT) and 2 Buehler tests.

The GPMT (Anonymous, 1986b) was performed in Hartley guinea pigs (20 males/group) according to the OECD TG 406 and GLP. The test material was technical grade (87.2%) esfenvalerate. Intradermal injections of 25% (esfenvalerate in corn oil) and topical applications of 100 % test material were used for induction, and 100 % was used for topical challenge. Topical induction was preceded by treatment with sodium lauryl sulphate, and occlusive dressing was used in both topical applications. In the esfenvalerate treated group, there was slight to moderate erythema in 15 out of 20 guinea pigs after 24 hours. Three animals also exhibited slight oedema. The number of animals with erythema increased to 17 (85%) after 48 hours. There was no response in the negative control animals. There were no treatment-related effects on body weight. The positive control (DNCB) caused moderate to severe skin sensitisation reactions in all animals. In the GPMT esfenvalerate was shown to be positive for skin sensitisation.

The first Buehler test (Anonymous, 1986c) was done according to the OECD TG 406 with deviations: the group size was 10 animals/group instead of 20, and 9 topical inductions were used instead of 3. The test material was technical grade (87.2%) esfenvalerate. Both induction and challenge used 100% test material, with occlusive dressing. There were no signs of erythema or oedema in animals treated with esfenvalerate. Positive control (DNCB) treated animals showed slight to moderate erythema and slight to severe oedema. No skin sensitisation was shown in the Buehler test.

The second Buehler test (Anonymous, 1986d) was done according to the OECD TG 406 with deviations: the group size was 10 animals/group instead of 20. The test material was technical grade esfenvalerate (purity not reported). There were no significant dermal reactions observed during the induction period in the animals treated with esfenvalerate. After the challenge applications no dermal reactions were observed in the animals treated with esfenvalerate. Positive control (2,4-DNCB) did produce evidence of hypersensitivity. No skin sensitisation was shown in the Buehler test.

The DS concluded that according to the GPMT esfenvalerate is a skin sensitiser, and proposed Skin Sensitisation Category 1, arguing that Category 1A cannot be excluded, as data are available from a test showing a high response after exposure to a high concentration but lower concentrations, which could show the presence of effects at lower doses, have not been tested.

Comments received during public consultation

Three MSCAs supported the classification proposed by the DS.

Assessment and comparison with the classification criteria

Based on the described GPMT, which was performed according to the OECD TG 406 and GLP, using 25% test material for intradermal induction, esfenvalerate is a skin sensitiser: in the esfenvalerate treated group, there was slight to moderate erythema in 15 out of 20 guinea pigs after 24 hours. Three animals also exhibited slight oedema. The number of animals with erythema increased to 17 (85%) after 48 hours. The Buehler tests did not show skin sensitising properties, but they did not use the proper number of animals (10 instead of 20/group) and this assay is less sensitive than the maximisation test.

According to the ECHA Guidance on the Application of the CLP Criteria, classification into subcategories is required when data are sufficient. The results from the GPMT suggest that classification in Category 1B may be appropriate, as ≥30% of the animals responded at >1% intradermal induction dose. However, when Category 1A cannot be excluded, Category 1 should be applied instead of Category 1B. This is particularly important if only data are available from certain tests showing a high response after exposure to a high concentration but where lower concentrations, which could show the presence of effects at lower doses, have not been tested.

In this case, only one intradermal induction concentration (25%) was investigated in the guinea pig maximisation test. Therefore, the possibility that sensitisation would have occurred at lower induction concentrations cannot be excluded. Therefore RAC agrees with the DS for **classification for skin sensitisation as Skin Sens. 1; H317: May cause an allergic skin reaction.**

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

Summary of the Dossier Submitter's proposal

There are 8 studies discussed in the CLH report for STOT RE: a 28 day dietary study in rats, two 90-day dietary studies in rats, two 90-day dietary neurotoxicity studies in rats, a 90-day dietary study in mice, a one-year oral study in Beagle dogs, and a 21-day dermal study in rats.

28 day dietary study

In the 28 day dietary study (Anonymous, 2008), performed according to OECD TG 407 (non GLP), esfenvalerate was given to Wistar (HanRcc: WIST (SPF)) rats (10 animals/sex/dose) in pelleted diet at a concentration of 0, 300, 500, 700 and 1000 ppm (0, 22, 35.4, 46 or 44 mg/kg bw/d for males and 0, 23.1, 39.8, 54, or 46.5 mg/kg bw/d for females) for 4 weeks. The test material was technical grade (87.3%) esfenvalerate. Cageside observations, food consumption and body weights were recorded and all animals were subjected to necropsy and post mortem examination.

At **1000 ppm** (44 mg/kg bw/d in males, 46.5 mg/kg bw/d in females) the dose is lower than in the 700 ppm group, which is due to the severe toxicity and significantly reduced food consumption by day 7, when the dosage was recorded. Between days 7 and 12 of treatment, 7 males were found dead. Two males in this group had to be killed in extremis on day 11 and the remaining male on day 12. Two females in the top dose group died spontaneously on day 7 and two further females on day 8. The remaining 6 females in this group had to be killed in extremis on day 8 for ethical reasons. Clinical signs found were ataxia (all animals), aggressive behaviour (1 female) and vocalisation when touched (1 female), prostration (1 female), abnormal/swaying gait and muscle twitching. Food consumption (71.8% in males, 56.2% in females) and body weight (23.6% in males, 21.3% in females) were reduced. Macroscopic findings were the following: dark red discoloured lungs (4 males, 2 females); dark red discoloured lungs and thymus (1 male).

At **700 ppm** (46.0/54.0 mg/kg bw/d) 1 male died spontaneously on day 28. Observed clinical signs were stiff gait (1 female), ataxia (5 females), abnormal/swaying gait and muscle twitching. Food consumption (26.8% in males, 9.6% in females) and body weight (19.7% in males, 14.0% in females) were reduced.

At **500 ppm** (35.4/39.8 mg/kg bw/d) the clinical signs were abnormal/swaying gait and muscle twitching. Food consumption (14.6% in males) and body weight (11.6% in males) were reduced.

At **300 ppm** (22.0/23.1 mg/kg bw/d) there were no toxicologically relevant findings (NOAEL).

90 day dietary study in rat I.

In the first 90 day dietary study (Anonymous, 1984) performed according to OECD TG 408, esfenvalerate was given in the diet to Sprague Dawley derived rats (30 animals/sex/dose), at dose levels of 0, 50, 150, 300 and 500 ppm (0, 2.5, 7.5, 15 or 25 mg/kg bw/d) for up to 13 weeks. The test material was technical grade esfenvalerate (purity not reported). After seven weeks exposure, up to 10 rats/sex/group were randomly selected and evaluated at an interim necropsy. After 13 weeks, up to five animals/sex/group were used for electron microscopy evaluations and the remaining animals sacrificed for post mortem examination.

At **500 ppm** (25.0 mg/kg bw/d, later recalculated as 34 mg/kg bw/d based on actual bodyweights) 4, 1 and 1 female rats died in weeks 6, 7 and 11, respectively, and 1 female was sacrificed in a moribund state in week 9. Observed clinical signs were jerky leg movements, unsteady gait, body tremors, hypersensitivity to sounds, convulsions, and the signs were usually observed from within the first few weeks of dosing to termination in this dose group. Body weight and food consumption were decreased significantly. Microscopic findings were slight to moderate hypertrophy of the parenchymal cells in the parotid salivary gland and with lower incidence in the pituitary glands.

At **300 ppm** (15.0 mg/kg bw/d) jerky leg movements, unsteady gait were observed and body weight and food consumption decreased significantly in males. Microscopic findings were slight to moderate hypertrophy of the parenchymal cells in the parotid salivary gland and with lower incidence in the pituitary glands.

At **150 ppm** (7.5 mg/kg bw/d) one animal exhibited jerky leg movements.

At **50 ppm** (2.5 mg/kg bw/d) there were no toxicologically relevant findings (NOAEL).

90 day dietary study in rat II.

In the second 90 day dietary study (Anonymous, 1987), performed according to OECD TG 408, esfenvalerate was mixed in the diet to five groups of Sprague Dawley derived rats (25 animals/sex/dose) at levels of 0, 75, 100, 125 and 300 ppm (0, 3.75, 5, 6.25 or 15 mg/kg bw/d) for either 7 (10 rats/sex/group) or 13 (15 rats/sex/group) weeks. The test material was technical grade esfenvalerate (purity not reported). No microscopic evaluation was performed, as no treatment related findings were noted below 300 ppm in the previous study.

At **300 ppm** (15 mg/kg bw/d) there were neurological signs beginning at week 10 of the study and characterised by hyperactivity and/or abnormal limb movements (jerky leg movements characterised by prolonged posterior extension, flexion, and/or elevation of one or both hind limbs). The late onset of these signs is atypical compared with other repeated dose studies at a similar dose level. Other findings were higher absolute and relative kidney weights.

At **125 ppm** (6.25 mg/kg bw) and below, there were no toxicologically relevant findings (NOAEL).

90-day dietary neurotoxicity study I.

In the first 90-day dietary neurotoxicity study (Anonymous, 2000c), performed according to OECD TG 424, Sprague Dawley rats (12 animals/sex/dose) were fed a diet containing 0, 50, 100 or 300 ppm (0, 3.2, 6.4/7.3 or 20.1 mg/kg bw/d) esfenvalerate. The test material was technical grade esfenvalerate (purity not reported). The observations included clinical examinations, functional observational battery (FOB) and motor activity measurements (pre-dose, weeks 4, 8 and 13). Neurohistopathology was conducted at termination. There were no treatment related macroscopic necropsy findings. The microscopic examination of the nervous system tissues did not reveal any treatment-related changes.

At **300 ppm** (20.1 mg/kg bw/d) there were 2 unscheduled deaths (males: killed early due to serious skin sores, on day 52 and day 88). Bodyweight was reduced (males: 12.5%, females 10%) as well as body weight gain (males: 19.3%, females: 20.7%). Food consumption was reduced in males (7%). Abnormal gait was observed in both males and females. There was a reduction in forelimb grip strength (in males at week 4 and 8; in females at week 4) and a reduction in hindlimb grip strength (in males at 4 weeks; in females at 4 and 13 weeks).

At a **100 ppm** (6.4/7.3 mg/kg bw/d) reduced body weight gain in males (10.4%) was recorded. Treatment-related reductions in forelimb grip strength were observed in males. (NOAEL female).

At **50 ppm** (3.2 mg/kg bw/d) there were no toxicologically relevant findings (NOAEL male).

90-day dietary neurotoxicity study II.

In the second 90-day dietary neurotoxicity study (Anonymous, 1999c), performed according to OECD TG 424, Sprague-Dawley (CD) rats (12 animals/sex/dose) were fed a diet containing esfenvalerate at dose levels of 0, 40, 120 or 360 ppm (0, 3.0, 8.9, or 28.8 mg/kg bw/d). The test material was technical grade (86.0%) esfenvalerate. A functional observational battery (FOB) both qualitative and quantitative –grip strength and hindlimb splay- and motor activity test were performed prior to treatment initiation and during weeks 2, 5, 9 and 13, and an ophthalmological examination was conducted prestudy and during week 13. At study completion, five rats/sex/group were given a whole-body perfusion (with brain dimensions later measured) and the animals in the control and high dose groups subsequently underwent a neuropathological examination. Various peripheral nerves, parts of the brain and brain-associated organs, parts of the spinal cord and muscles were examined. There were no treatment related mortalities. The only clinical signs attributed to treatment were observed in a small number of 360 ppm group males, which showed lesions/scabbing at the inguinal/sacral/urogenital/scrotal regions.

At **360 ppm** (28.8 mg/kg bw/d) skin ulcerations (males) and reduced body weight (males and females) were recorded. Forelimb grip strength was significantly reduced (males and females), and there was reduced ease of removal from home cage (females only) at week 2. Significant decrease in total activity counts was observed (females only) at week 2.

At **120 ppm** (8.9 mg/kg bw/d) significant decrease in total activity counts (females only) were observed at week 2.

40 ppm (3.0 mg/kg/day) there were no toxicologically relevant findings (NOAEL).

90 day dietary study (mouse)

In the 90 day dietary study in mice (Anonymous, 1985h) performed according to the OECD TG 408, B6C3F1 mice (12 animals/sex/dose) were fed diets containing 0, 50, 150 or 500 ppm (0, 10.5, 30.5, or 106/113 mg/kg bw/d) esfenvalerate. The test material was technical grade (87.2%) esfenvalerate.

At **500 ppm** (106/113 mg/kg/d) a reduction of body weight gain was noted for males (-51 %) and females (-35 %). Treatment related clinical signs included fibrillation, tremor, convulsion, hypersensitivity to sounds (during early stage of the study), abnormal gait (hunched posture and unsteady gait), salivation (week 1 of the study), higher grooming activities such as scratch and licking, leading to higher incidence of external lesions such as alopecia, scab and sore formation. Changes in clinical pathology parameters included anaemia and altered plasma lipid parameters. Histopathological findings included inflammatory changes in skin; reactive changes in lymphatic tissues, slight ulcerative changes in stomach and decrease of fat deposition in liver and kidneys (correlated with lower plasma lipids).

150 ppm (~30.5/36.5 mg/kg/d) there were no toxicologically relevant findings (NOAEL).

One-year oral study in dogs

In the one-year oral study in dogs (Anonymous, 1986e) performed according to OECD TG 452, esfenvalerate (technical grade, purity not reported) was fed to Beagle dogs (6 animals/sex/dose) at dose levels of 0, 25, 50, 100, 200 ppm (0, 0.66, 1.28, 2.58, 5.02 mg/kg bw/d). No signs of toxicity were observed during the study and there were no mortalities. There were no treatment-related effects on mean body weight, mean food consumption, ophthalmic examination, organ weights or macroscopic and microscopic findings. Differences noted between treated and control animals in clinical pathology parameters were considered to be normal biological variations. NOEL was 200 ppm (~5 mg/kg/d).

21 day dermal study in rats

In the 21 day dermal study in rats (Anonymous, 2000b), done according to OECD TG 410, Sprague-Dawley rats (10 animals/sex/dose) were exposed to dermal doses of 0, 25, 125, 500 and 1000 mg/kg bw/day of esfenvalerate. Standard investigations were conducted. A comprehensive functional observation battery (FOB) and motor activity measurements were conducted on all animals prior to exposure and during week 3. There were no treatment related deaths, or adverse effects on bodyweight or food consumption. FOB did not reveal any treatment-related effects. There were no treatment related haematology or clinical chemistry findings, organ weight differences, macroscopic or microscopic pathology findings.

At **1000 mg/kg bw/day,** during the 1st week, abnormal hind limb gait was observed in all animals, vocalisation was reported for most females, predominantly during the first 3 days of dosing. Most females exhibited hyperactivity at the start of the study and hyperreactivity at other times. Vocalisation, hyperactivity and hyperreactivity may be secondary to the skin sensory stimulation previously reported in both humans and animals, rather than due to direct systemic toxicity. The motor activity assessment at week 3 showed increased activity in comparison with baseline and control activity levels, measured as duration of movements and number of movements during the 60 min observation period, among females at 500 and 1000 mg/kg/day. The increased activity may be secondary to skin sensory stimulation.

At **500 mg/kg bw/day,** during the 1st week, abnormal hind limb gait was observed in all animals, vocalisation was reported for most females predominantly during the first 3 days of dosing.

At **125 mg/kg/day** abnormal hind limb gait was observed in 50% of males and all females.

A NOAEL of **25 mg/kg bw/d** was identified.

Conclusion

Oral route

The DS considered only the rat data relevant for classification purposes, as in mice and dogs, no significant signs of toxicity were observed at doses relevant for classification. The DS pointed out that treatment-related neurological effects observed in repeated dose toxicity studies via the oral route were typical of those observed after acute exposure. There were no significant neuropathological changes and there was no increase in the incidence or severity of neurological effects with time in short term and chronic studies. The neurological effects in the rat were generally observed at dose levels ≥15 mg/kg/bw/d (effective dose). According to the DS, where the same target organ toxicity of similar severity is observed after single and repeated exposure to a similar dose, it may be concluded that the toxicity is essentially an acute (i.e. single exposure) effect with no accumulation or exacerbation of the toxicity with repeated exposure. Therefore, the DS concluded that the neurological effects seen in the repeated dose studies do not warrant

classification for STOT RE, as they are already covered by the classification for acute toxicity (Acute Tox. 3; H301).

However, in a 28 day study all animals in the top dose group (1000 ppm, equivalent to 44.0 and 44.5 mg/kg bw/d in males and females, respectively) had died or were killed in extremis by day 12, and in one of the 90 day studies 7 females died in the top dose group (25 mg/kg bw/d) between 6 and 11 weeks of treatment. The cause of the deaths was not determined during the study, although convulsions were noted in the animals that subsequently died. The DS considered that in both of these studies, the deaths occurred too late to be considered to be an acute effect, and in addition in the acute oral toxicity study, deaths were only observed at doses ≥ 55 mg/kg bw (the LD_{50} was 88.5 mg/kg bw in both sexes). In the opinion of the DS, the timings of death are consistent with a repeated dose effect, rather than acute toxicity; therefore on the basis of deaths occurring at 25 mg/kg bw/d in a 90 day study, the DS proposed classification in STOT RE 2; H373: May cause damage to organs through prolonged or repeated exposure.

Dermal route

In a 21-day dermal toxicity study in rats, abnormal hind limb gait typical of that seen in acute oral (neuro)toxicity studies was observed during the first week at ≥125 mg/kg/d but not during the remainder of the study, while there were no other systemic neurological signs. The effect was not considered to be of sufficient severity to warrant STOT RE classification.

Comments received during public consultation

Three comments from MSCAs were received during the public consultation: all agreed with the DS's proposal to classify esfenvalerate as STOT RE 2, but one was of the opinion that classification a STOT RE (nervous system) should be discussed based on studies that showed neurological effects at dose levels which fulfil the criteria for a classification in category 2.

The DS replied that the treatment-related neurological effects observed in repeated dose toxicity studies via the oral route were typical of those observed after acute exposure. There were no significant neuropathological changes and there was no increase in the incidence or severity of neurological effects with time in short term and chronic studies. The neurological effects in the rat were generally observed at dose levels ≥15 mg/kg bw/d (effective dose). The DS pointed out that where the same target organ toxicity of similar severity is observed after single and repeated exposure to a similar dose, it may be concluded that the toxicity is essentially an acute (i.e. single exposure) effect with no accumulation or exacerbation of the toxicity with repeated exposure (ECHA Guidance on the Application of the CLP Criteria, 2017). Therefore, the DS concluded that the neurological effects seen in the repeated dose studies do not warrant classification for STOT RE, as they are already covered by the classification for acute toxicity (Acute Tox 3; H301).

Assessment and comparison with the classification criteria

Specific target organ toxicity (repeated exposure) means specific, target organ toxicity arising from a repeated exposure to a substance or mixture. All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed are included. However, specific toxic effects covered by other hazard classes are not included in STOT RE.

Oral route

There are 9 studies that can be taken into consideration via the oral route: two studies in mice (a 90-day dietary study and an 18 month carcinogenicity study), a one-year oral study in Beagle dogs, and 6 studies in rats (28 day dietary study, two 90-day dietary studies, two 90-day dietary neurotoxicity studies, and a 2 year combined chronic toxicity/carcinogenicity study).

Mice

90-day dietary study: B6C3F1 mice (12 animals/sex/dose) were fed diets containing 0, 50, 150 or 500 ppm (0, 10.5, 30.5, or 106/113 mg/kg bw/d) esfenvalerate. The highest dose was equivalent to 106 mg/kg bw/d in males and 113 mg/kg bw/d in females. The middle dose of 150 ppm was identified as NOAEL. There were no treatment-related effects observed at doses relevant for classification ($10 < C \leq 100$ for category 2).

In the **carcinogenicity study**, all mice at the top dose (350 ppm) were sacrificed by day 58 due to excessive self trauma induced by the powdered test substance on dermal sensory nerves. A large number of mice were also sacrificed early in the 150 ppm (18.3 mg/kg bw/d) dose group due to self-mutilation. The dose level relevant for classification (35 ppm: 4.29 mg/kg bw/d) was identified as the NOAEL.

Dogs

There were no treatment-related findings observed in dogs following oral administration of doses up to 5.0 mg/kg bw/d esfenvalerate for one year.

Rats

There are two significant or severe health effects that can be identified in the rat studies at dose levels relevant for classification: neurotoxic effects and mortality.

Neurotoxicity

In all four 90 day dietary/dietary neurotoxicity studies, dose-related neurological effects were observed at dose levels (≤ 100 mg/kg bw/d) relevant for classification.

In the **first 90 day dietary study** (Anonymous, 1984), at 500 ppm (25.0 mg/kg bw/d, later recalculated as 34 mg/kg bw/d based on actual bodyweights) the observed clinical signs were jerky leg movements, unsteady gait, body tremors, hypersensitivity to sounds and convulsions. These signs were usually observed from within the first few weeks of dosing to termination in this dose group. At 300 ppm (15.0 mg/kg bw/d) jerky leg movements and unsteady gait were observed.

In the **second 90 day dietary study** (Anonymous, 1987), at 300 ppm (15 mg/kg bw/d) there were neurological signs beginning at week 10 of the study and characterised by hyperactivity and/or abnormal limb movements (jerky leg movements characterised by prolonged posterior extension, flexion, and/or elevation of one or both hind limbs). The late onset of these signs is atypical compared with other repeated dose studies at a similar dose level.

In the **first 90-day dietary neurotoxicity study** (Anonymous, 2000c), at 300 ppm (20.1 mg/kg bw/d) abnormal gait was observed in males and females. There was a reduction in forelimb grip strength (in males at week 4 and 8; in females at week 4) and a reduction in hindlimb grip strength (in males at 4 weeks; in females 4 and 13 weeks).

In the **second 90-day dietary neurotoxicity study** (Anonymous, 1999c), at 360 ppm (28.8 mg/kg bw/d) forelimb grip strength was significantly reduced (males and females), and there was reduced ease of removal from home cage (females only) at week 2. Significant decrease in total activity counts was observed (females only) at week 2.

In the **28 day dose range finding study** all animals (males and females) in the top dose group of 1000 ppm (44.0/46.5 mg/kg bw/d) died or were killed in extremis by day 12 of treatment. Clinical signs found were: ataxia (all animals), aggressive behaviour (1 female) and vocalisation when touched (1 female), prostration (1 female), abnormal/swaying gait and muscle twitching. At 700 ppm (46.0/54.0 mg/kg bw/d) the observed clinical signs were stiff gait (1 female), ataxia (5 females), abnormal/swaying gait and muscle twitching. At 500 ppm (35.4/39.8 mg/kg bw/d) the clinical signs were abnormal/swaying gait and muscle twitching.

In the 2 year combined chronic toxicity/carcinogenicity study (Anonymous, 2011a) a FOB and locomotor activity (60 min time period) measurements were conducted at week 48 in satellite animals. A significant reduction in hindlimb grip strength was noted in both sexes at the top dose (18.5/21.5 mg/kg bw/d: above the dose level relevant for classification), and there were no related histopathological findings in skeletal muscle, sciatic nerve and lumbar spinal cord.

The effects seen in the repeated dose studies are similar to those noted in the acute toxicity studies, at similar dose levels:

In the acute oral toxicity study at 10 mg/kg bw transient muscular fibrillation and decreased spontaneous activity, and at 40 mg/kg bw transient muscular fibrillation, occasional signs of tremor, limb paralysis and ataxia were observed (Anonymous, 1985d). In one **acute oral neurotoxicity study** (Anonymous, 2000a), stereotypical grooming and tremors were seen in occasional animals at 20 mg/kg bw, and at 80 mg/kg bw salivation, tremors, un-coordination, stereotypical grooming, abnormal gait, paw shaking in both genders, slow righting reflex and increased reaction to touch or tail pinch, reduced motor activity, reduced forelimb grip strength and hind limb foot splay were observed. In another **acute oral neurotoxicity study** (Anonymous (1985e), clinical signs of toxicity such as muscular fibrillation, hunched posture and ataxia were noted in the intermediate (20 mg/kg bw) and high (90 mg/kg bw) dose groups.

In the repeated dose studies the effects started early during treatment (except for one neurotoxicity study), and the severity and incidences of findings did not increase with duration, only with dose. There are no histopathological alterations in any of the studies in relation to functional effects. The neurological effects in the rat were generally observed at dose levels ≥15 mg/kg bw/d (effective dose).

According to the Guidance on the Application of the CLP Criteria, where the same target organ toxicity of similar severity is observed after single and repeated exposure to a similar dose, it may be concluded that the toxicity is essentially an acute (i.e. single exposure) effect with no accumulation or exacerbation of the toxicity with repeated exposure. Therefore RAC proposes that the neurological effects seen in the repeated dose studies **do not warrant classification as STOT RE**, as they are already covered by the classification as STOT SE 1 H370 (nervous system).

Mortality

In the **28 day dietary study** (Anonymous, 2008), at the top dose of 1000 ppm (44/46.5 mg/kg bw/d) between days 7 and 12 of treatment, 7 males were found dead, two had to be killed in extremis on day 11 and the remaining male on day 12. Two females died spontaneously on day 7 and another two on day 8. The remaining 6 females in this group had to be killed in extremis on day 8 for ethical reasons. At 700 ppm (46.0/54.0 mg/kg bw/d) 1 male died spontaneously on day 28. The cause of death of these animals is not clear. In a **90 day dietary study** (Anonymous, 1984) at 500 ppm (25.0 mg/kg bw/d, later recalculated as 34 mg/kg bw/d based on actual bodyweights) 4, 1 and 1 female rats died in weeks 6, 7 and 11, respectively, and 1 female was sacrificed in a moribund state in week 9. Gross and microscopic evaluations did not reveal the cause of death, although convulsions were noted in the animals that subsequently died. No mortality occurred in the other **90 day dietary study** (Anonymous, 1987), where 300 ppm (15

mg/kg bw/d) was the top dose. At 300 ppm (20.1 mg/kg bw/d) in the **90-day dietary neurotoxicity study** (Anonymous, 2000c), there were 2 unscheduled deaths (males: killed early due to serious skin sores, on day 52 and day 88. There were no treatment related mortalities reported in the other **90-day dietary neurotoxicity study** (Anonymous, 1999c), where 360 ppm (28.8 mg/kg bw/d) was the top dose.

The doses at which the mortalities occurred were 44/46.5 mg/kg bw/d in the 28 day study and 34 mg/kg bw/d in the 90 day study, both at dose levels which are relevant for classification in STOT RE 2 ($30 < C \leq 300$ and $10 < C \leq 100$ respectively). In the acute oral toxicity studies deaths were observed at doses ≥ 55 mg/kg bw. Although the doses causing mortality in the single and repeated studies are close to each other, in the studies where mortality occurred, the deaths were reported after one week (28 day study) or after 5 weeks (90 day study), and therefore too late to be considered to be an acute effect.

On the basis of the above reasoning, RAC supports the DS's proposal to classify esfenvalerate as **STOT RE 2; H373: May cause damage to organs through prolonged or repeated exposure**.

Dermal route

In a **21-day dermal toxicity study** in rats, effects seen at dose levels relevant for classification ($80 < C \leq 800$) were abnormal hind limb gait during the first week at ≥125 mg/kg bw/d but not during the remainder of the study, and vocalisation in females at ≥500 mg/kg bw/d primarily for the first 3 days of dosing. RAC agrees with the DS that these effects are **not sufficiently severe to warrant classification as STOT RE via the dermal route**.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

Five tests are included in the CLH dossier: a bacterial reverse mutation test, performed according to the OECD TG 471 (Kogiso, 1985a), an in vitro chromosome aberration test, performed according to the OECD TG 473 (Kogiso, 1985b), an in vitro mammalian cell mutation test performed according to the OECD TG 476 (Kogiso, 1985c), an in vitro UDS test performed according to the OECD TG 482 (Kogiso, 1986) and an in vivo bone marrow micronucleus test performed according to the OECD TG 474 (Anonymous, 1985g). The test material was technical grade (87.2%) esfenvalerate in all cases. Deviations: in some studies the positive control substances differed from those recommended in the guideline. Nevertheless, in all studies positive controls behaved as expected. In some studies Kanechlor 400 was used instead of Aroclor 1254 in the preparation of S-9 mix. Esfenvalerate was found to be negative in all the studies.

The DS concluded that the genotoxicity of esfenvalerate has been adequately investigated in standard tests, which were all negative, therefore no classification is proposed.

Comments received during public consultation

One MSCA agreed to not classify esfenvalerate for mutagenicity. One MSCA would have liked to see concrete data (frequencies etc.) in the mutagenicity result table. The DS replied that the results were clearly negative, however, provided the study reports as a confidential attachment in case RAC finds them useful to their assessment.

Assessment and comparison with the classification criteria

The genotoxicity of esfenvalerate has been adequately investigated in battery of standard tests. It was found negative in *in vitro* assays for gene mutations (bacterial reverse mutation and mammalian cell mutation), clastogenicity and unscheduled DNA synthesis. The substance was also negative in an *in vivo* micronucleus test. It is therefore concluded that esfenvalerate is not genotoxic. RAC supports the DS's proposal **not to classify for germ cell mutagenicity.**

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

Two studies were included in the CLH dossier: a 2 year combined chronic toxicity/ carcinogenicity study in rats, and an 18 month dietary study in mice. In addition, a comprehensive suite of studies has been conducted to investigate the endocrine disrupting potential of esfenvalerate as part of the US EPA's Endocrine Disruptor Screening Program.

2 year combined chronic toxicity/ carcinogenicity study in rats

The study (Anonymous, 2011a) was performed according to OECD TG 453 and GLP. Wistar rats (70 animals/sex/dose) were fed a diet containing esfenvalerate (pelleted) at a concentration of 0, 15, 50, 150 or 400 ppm (~0.7, 2.3, 6.9, 18.5 mg/kg/bw d for males and 0.8, 2.7, 8.0 and 21.5 mg/kg/bw d for females). The test material was technical grade (87.3%) esfenvalerate. The dose levels were selected based on the results of 28 day and 90 day feeding studies in rats, the top dose (400 ppm) was selected based on signs of toxicity including deaths seen at 500 ppm and above in these studies. 50 rats/sex/dose were used for the main study (sacrificed after 104 weeks) and 20 rats/sex/dose were used as a satellite group, sacrificed after 52 weeks.

<u>Non-neoplastic findings</u>

There were no treatment-related clinical signs, or effects on survival rates. Body weights were reduced in treated males; the effect was statistically significant at the top dose only (mean body weights in this dose group were 9.7% lower than controls at study termination).

In satellite animals a FOB and locomotor activity (60 min time period) measurements were conducted at week 48. A significant reduction in hindlimb grip strength was noted in both sexes at the top dose, but forelimb grip strength was not affected, and there were no related histopathological findings in skeletal muscle, sciatic nerve and lumbar spinal cord. There was a dose-related decrease in total activity among treated males, however, the effect was not statistically significant.

The number of males at 400 ppm (main study) with spinal cord radiculoneuropathy was significantly increased, but the incidences were within the historical control range. In addition, there was no relationship between radiculoneuropathy and the presence of clinical signs or histopathological changes in the peripheral nerve, central nervous system or skeletal muscle. Overall, the radiculoneuropathy is not considered to be a severe lesion and is considered to be an age-related incidental finding.

There were no treatment-related ophthalmoscopy, haematology, clinical chemistry or urinalysis findings, and no organ weight changes or macroscopic findings that were considered to be treatment-related. In terms of microscopic findings, there were no treatment-related non-neoplastic effects.

Neoplastic findings

In the control and top dose groups, all animals were examined (i.e., decedent and survivors). For the 15, 50 and 150 ppm groups, only the animals with gross lesions or those found dead were subject to histopathological investigation. The individual tumour types with an incidence in any treatment group that was statistically significantly higher than the current control group is shown in *Table 1*. In a later study (Anonymous 2015) a histopathological examination of the testes in all animals of the intermediate dose groups was conducted to clarify the total incidence of Leydig cell tumours (*Table 2*.).

Table 1. *Tumour types showing statistically significant differences, incidence (no. of affected animals/no. examined) in main study animals, decedents and survivors combined.*

Tissue & tumour type	Dietary concentration of esfenvalerate TG (ppm)									
	Males					Females				
	0	15	50	150	400	0	15	50	150	400
Testes: Leydig cell tumour (benign)	2/50 [4%]	1/27 [4%]	0/17 [0%]	4*/15 [27%]	4/50 [8%]					
Lab. historical control	17/628 [2.7%, range 0-4%]									
Pituitary gland: adenoma pars anterior	8/50 [16%]	17†/27 [63%]	10†/18 [56%]	10†/16 [62%]	13/50 [26%]	20/50 [40%]	23*/35 [66%]	25†/31 [81%]	25†/34 [73%]	21/50 [42%]
Lab. historical control	210/626 [33.5%, range 28.0-38.9%]					349/624 [55.9%, range 42.0-71.3%]				
Parathyroid glands: adenoma	0/38 [0%]	0/17 [0%]	1/12 [8%]	2*/11 [18%]	1/42 [2%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]
Lab. historical control	8/532 [1.5%, range 0-5.1%]					1/550 [0.2%, range 0-1.2%]				
Thymus; thymoma lymphatic type, benign	0/47 [0%]	1/22 [4%]	2*/14 [14%]	1/15 [7%]	1/45 [2%]	0/48 [0%]	6†/25 [24%]	5†/23 [22%]	5†/17 [29%]	3/49 [6%]
Lab. historical control	9/600 [1.5%, range 0-4.4%]					22/615 [3.6%, range 0-16%]				
Haemolymphoreticular system: malignant lymphoma	2/50 [4%]	3/24 [12%]	1/12 [8%]	1/11 [9%]	2/50 [4%]	0/50 [0%]	3*/17 [18%]	0/19 [0%]	0/14 [0%]	0/50 [0%]
Lab. historical control	9/480 [1.9%, range 0-3.7%]					4/480 [0.8%, range 0-2.0%]				
Mammary gland: fibroadenoma	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	10/50 [20%]	14†/27 [52%]	19†/29 [66%]	13†/23 [57%]	13/49 [27%]
Lab. historical control	0/479 [0%, range 0-0%]					180/626 [28.8%, range 22.0-36.0%]				
Mammary gland: adenocarcinoma	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	5/50 [10%]	3/27 [11%]	6/29 [21%]	7*/23 [30%]	5/49 [10%]
Lab. historical control	1/479 [0.2%, range 0-2%]					37/626 [5.9%., range 2.0-12.0%]				

* significantly different from control, p<0.05 † significantly different from control, p<0.01

Historical control data is from 6-8 chronic (104 week) toxicity studies conducted at Harlan Laboratories in Wistar rats, completed between July 2005 and February 2009

The overall incidence of animals with benign and/or malignant tumours was similar in all groups.

The percentage of animals in the 15, 50 and 150 ppm groups with tumours of the pituitary gland (males and females), the haemolymphoreticular system (males), thymus (females) and mammary gland (females) was noticeably higher than both the control group and the 400 ppm group. This is due to the fact that for the 15, 50 and 150 ppm groups only the animals with gross lesions or those found dead were subject to histopathological examination, and is not interpreted as evidence of a monotonic dose-response relationship. In each case, there is no coherent dose-response relationship over the range 15 to 400 ppm and the DS concluded that there is no

evidence of carcinogenic activity in the pituitary, the haemolymphoreticular system, thymus or mammary gland.

Table 2. *The results of the histopathological examination of the testes in all animals.*

	Dietary concentration of esfenvalerate TG (ppm)				
	0	**15**	**50**	**150**	**400**
No. of animals examined	50	50	50	50	50
Leydig cell hyperplasia	2	1	0	1	0
Leydig cell tumour	2	1	0	4	4

The percentage of animals (male) with benign Leydig cell tumours was greater than controls in the 150 and 400 ppm treatment groups in the original study. The effect was statistically significant at 150 ppm only. The evaluation of additional testes sections (*Table 2.)* from animals of intermediate groups (15, 50 and 150 ppm) that were not evaluated during the main study did not reveal new preneoplastic or neoplastic lesions. The revised % incidence of benign Leydig tumours was therefore 4%, 2%, 0%, 8% and 8% in the control, 15, 50, 150 and 400 ppm groups respectively, and the incidence of Leydig cells hyperplasia was 2%, 1%, 0%, 1% and 0% in the control, 15, 50, 150 and 400 ppm groups respectively.

According to the DS, the incidence of benign Leydig cell tumours at the top two doses was greater than controls, but there was no clear dose-response, and the difference compared to controls was not statistically significant. Historical control data from the same laboratory show that the incidence in control animals was 0-4% over the 5 years prior to the study being conducted. The DS argued that although older historical control data should be treated with caution, historical control incidences of 9.1, 10.0 and 10.0% were reported in the same laboratory 14, 17 and 22 years prior. In addition, there was no temporal trend in the background incidence of Leydig cell tumours, which supports the comparison with the control incidences between 1983 and 2004.

The DS further pointed out that there was no treatment-related increase in the incidence of Leydig cell hyperplasia (% incidence: 4, 2, 0, 2, 0), and no malignant tumours were reported at any dose level. Furthermore, in the available repeated dose toxicity studies and reproductive toxicity studies on esfenvalerate, there were no findings which were indicative of an adverse effect on the testes or the endocrine system. Overall, the DS proposed that the slight increase in benign Leydig cell tumours seen at the top two doses in the rat carcinogenicity study are not treatment-related, not statistically or biologically significant, and are therefore not relevant for classification.

18 month dietary study in mice

The study (Anonymous 1997) was done according to the OECD TG 451. Crl: CD mice (80 animals/sex/dose) were fed diets containing 0, 35 or 150 ppm (0, 4.3, 18.3, mg/kg/d) of esfenvalerate (powdered) for 18 months. The test material was technical grade (84.8%) esfenvalerate. An additional group received 350 ppm but developed excessive morbidity and mortality due to self-trauma induced by the powdered test substance on dermal sensory nerves and were sacrificed by design on test days 57 and 58. In the 150 ppm group survival was significantly decreased in males (46%, compared to 70% in controls) and females (41%, compared to 71% in controls), largely attributable to the number of mice sacrificed "in extremis" following self-trauma. Survival of animals fed diets containing 35 ppm of the test substance was comparable to controls.

Reduced body weight gains were observed at 150 ppm (19% in males and 22% in females by the end of the study). Mean body weights were also reduced in this group (7% in males and 9% in females). The observed depression in mean body weight and mean body weight gain was interpreted to be due to the interplay of increased incidence and severity of dermal self-trauma and mild systemic toxicity.

Overall, there was no significant treatment-related effect on food consumption. Males and females in the 150 ppm group had moderately (24% - 47%) lower food efficiency values during the 0 - 56 day interval. Food efficiency values of treated groups were generally comparable to controls, however, for the last 15 months of the study. The lower food efficiency observed during the first few months of the study was interpreted to be the result of the additive effects of self-trauma and systemic toxicity.

The test substance-related increased incidences of gross and microscopic findings in the skin, ears, and eyes of males and females in the 35 and/or 150 ppm groups were due to self-trauma induced by the effects of powdered esfenvalerate and were considered not to be a target organ toxicity. No other treatment-related toxicological effects were reported during this study.

No treatment-related tumours were reported.

Mechanistic studies

A comprehensive battery of **mechanistic studies** were conducted on esfenvalerate as part of the US EPA's 'Endocrine Disruptor Screening Program' (*Table 3.*). All of the mechanistic studies were negative, and the DS pointed out that they do not provide any evidence of a carcinogenic potential of esfenvalerate.

Table 3. *Mechanistic studies on esfenvalerate.*

Type of study, guideline	Test substance, purity	Relevant information about the study (as applicable)	Observations
Rat (SLC:Wistar) 26-week dietary hormonal study in males Anonymous (1999a)	Esfenvalerate TG, (86%)	Dose levels: fenvalerate 0, 50, 150, 500, 1500 ppm (2.5, 7.6, 25.4, 74.6 mg/kg/d); esfenvalerate 375 ppm (18.7 mg/kg/d), 8 M/group. Blood samples at 4-week intervals for analysis of serum luteinizing hormone and testosterone concentrations	No treatment related effects on serum luteinizing hormone and testosterone concentrations with either esfenvalerate or fenvalerate
Rat (Sprague-Dawley), 10-day Hershberger bioassay for detecting androgenic activity, OECD 441 Anonymous (2011b)	Esfenvalerate TG, (85.7%)	Dose levels (oral gavage): 0, 3, 6, 9 mg/kg/d Anti-androgenic assay: co-administration of testosterone propionate at 0.4 mg/kg/d by subcutaneous injection Positive controls: testosterone propionate and flutamide	No treatment related changes in endocrine / reproductive organ weights (androgenic or anti-androgenic activity) The positive controls behaved as expected.
Rat (Sprague-Dawley), pubertal development and thyroid function in intact juvenile / peripubertal males, U.S. EPA, OPPTS 890.1500 Anonymous (2012a)	Esfenvalerate TG, (85.7%)	Dose levels (oral gavage): 0, 3, 9 mg/kg/d from post natal day (PND) 23 to 53/54	No treatment related effects on pubertal development, on serum levels of T4, TSH or testosterone, or on endocrine / reproductive organ weights and histopathology
In vitro estrogen receptor transcriptional	Esfenvalerate TG, (85.7%) (vehicle: acetonitrile)	Esfenvalerate was tested for its ability to act as an agonist of the human estrogen receptor alpha (hERα) using the hERα-HeLa-	No agonist activity The positive control behaved as expected.

Type of study, guideline	Test substance, purity	Relevant information about the study (as applicable)	Observations
activation, OECD 455 Anonymous (2012b)		9903 cell line. Concentrations: 10-10.6 to 10-3.6M Positive control: 17β-estradiol in DMSO	
In vitro H295R steroidogenesis assay, OECD 456 Anonymous (2012c)	Esfenvalerate TG, (85.7%) (vehicle: acetonitrile)	Esfenvalerate was tested for its potential to interact with the steroidogenic pathway beginning with the sequence of reactions occurring after the gonadotropin hormone receptors (FSHR and LHR) through the production of testosterone and estradiol/estrone via the human cell line H295R Steroidogenesis Assay. Concentrations: 0.0001 to 100 µM Positive controls: forskolin and prochloraz (in DMSO)	No induction or inhibition of steroid biosynthesis up to the limit of solubility in this assay (no treatment related changes in hormone levels) The positive controls behaved as expected.
In vitro aromatase inhibition using human recombinant microsomes, U.S. EPA, OPPTS 890.1200 Anonymous (2011c)	Esfenvalerate TG, (85.7%)	Esfenvalerate was tested for its ability to inhibit human recombinant microsomal aromatase activity, an enzyme responsible for the conversion of androgens to estrogens. Concentrations: between 1 x 10-10 and 2.5 x 10-5 M Positive controls: 4-hydroxyandrostenedione (4-OH ASDN) and radiolabelled ASDN ([1β-3H]-Androst-4-ene-3,17-dione, [3H]-ASDN (26.3 Ci (0.974 TBq)/mmol): radiochemical purity 99.972%	No significant inhibition of aromatase activity up to the limit of solubility The positive controls behaved as expected

Conclusion

In the DS's opinion the available data do not provide any evidence that esfenvalerate is carcinogenic: the slight increase in the incidence of benign Leydig cell tumours at the top two doses in the rat 2 year study was not statistically or biologically significant, and the 18 month mouse study did not find any neoplastic changes. Also, esfenvalerate was negative in standard in vitro and in vivo tests for genotoxicity, as well as in a range of mechanistic studies conducted to investigate the endocrine disrupting potential of esfenvalerate. Therefore the DS proposed no classification for carcinogenicity.

Comments received during public consultation

One MSCA proposed that classification as Carc. 2 should be discussed, as the rat chronic toxicity study revealed a higher incidence of Leydig cell tumour at the 2 highest doses. Although the incidence was not significantly increased, it exceeded the value of the historical control data (range of 0.0 – 4.0 calculated between 2005 to 2011). The MSCA objected to the use of historical control data older than 5 years prior to the conducted study. The MSCA also emphasized that the tested doses were very low (0, 0.7, 2.3, 6.9 and 18.5 mg/kg bw/d respectively for 0, 15, 50, 150 and 400 ppm). In the second chronic toxicity study performed in mice, no treatment-related tumours were noted, but as survival was significantly decreased in both sexes (high number of

mice sacrificed in extremis due to self-trauma), only a small number of animals survived to the end of the study and the presence or absence of tumours is thus difficult to analyse and conclude.

The DS replied that in their opinion, the slight increase in benign Leydig cell tumours in the 2 year combined chronic toxicity/ oncogenicity study using Wistar rats is not biologically significant, and is therefore not relevant for classification. The finding is for one tumour type (benign) in one species (the rat but not mouse) and occurred in one study with esfenvalerate, within biological variation. The incidences of benign Leydig cell tumours are within the historical control range of the laboratory and the published range for Wistar rats. There were no significantly increased pre-neoplastic changes (Leydig cell hyperplasia) in the rat 2 year study. From the results of multi-generational reproductive toxicity studies, esfenvalerate did not exhibit any evidence of known modes of action for testicular Leydig cell tumourigenicity via endocrine mediated effects.

A second MSCA stated that the incidences of benign Leydig cell tumours at 150 and 400 ppm (8%) were outside the range of the historical control data collected in the same laboratory during the 5 years prior to the study being conducted. They suggested that although esfenvalerate was negative in standard in vitro and in vivo tests for genotoxicity and tested negative in a range of mechanistic studies conducted to investigate the endocrine disrupting potential of esfenvalerate, not all potential modes of action with relevance to humans can be ruled out. In their opinion, the mechanism of action has not been sufficiently clarified and therefore the relevance for humans still remains unclear. Moreover, there was not a confounding effect of excessive toxicity at the top two doses where the incidence of benign Leydig cell tumours increased. There were no treatment-related clinical signs, or effects on survival rates. Body weights were reduced in treated males; the effect was statistically significant at the top dose only (mean body weights in this dose group were 9.7% lower than controls at study termination). It is possible that with higher doses tested, the increase in tumours could have been much greater. All the considerations mentioned reduce considerably the concern and it might be possible that the benign tumours in benign Leydig cell tumours male rats were chance observations. However, in their opinion a treatment-related tumour response cannot be excluded.

A third MSCA wrote that dose response relationship for benign Leydig cell tumours should be supported by suitable statistical analyses (e.g. trend testing or BMD), and a Cochrane-Armitage linear trend test without correction for survival results in a p value of 0.1475 (two-sided), supporting the DS interpretation that the statistically significant finding at 6.9 mg/kg bw/d may be due to chance.

The fourth MSCA stated that as there was no increase of Leydig cell hyperplasia, no malignant Leydig cell tumours and no dose-response they agree that the findings do not fulfil criteria for classification. However, since the study summaries on reproductive toxicity referred to are not available in Annex I and since the study summaries on RDT do not state if the testis actually was investigated, it is not possible to conclude if these result support the conclusion that effects lack biological significance. With respect to other tumour frequencies observed in animals with gross lesions or found dead, the only remaining concern following a correction for 50 animals/dose is an increase of benign thymoma in females. Although within the range 0-16% of the HCD stated, the incidences are well above the concurrent control and the mean value of 3.6% in the HCD. However, considering the benign nature of this tumour type, which was only observed in females, the lack of dose-response and the lack of other types of tumours, the criteria for classification are not considered fulfilled. Therefore, overall the MSCA agreed that the data on esfenvalerate does not fulfil criteria for classification.

Assessment and comparison with the classification criteria

Two chronic dietary studies are available: a 2 year combined chronic toxicity/ carcinogenicity study (OECD 453, GLP) in rats, and an 18 month dietary study (OECD 451) in mice.

In the **18 month dietary study, mice** (80 animals/sex/dose) were fed diets containing 0, 35, 150 ppm (0, 4.3, 18.3 mg/kg/d) of powdered esfenvalerate for 18 months. Mice in the 350 ppm group developed excessive morbidity and mortality due to self trauma induced by the effects of the powdered test substance and were sacrificed by design on test days 57 and 58. In the 150 ppm group survival was significantly decreased in males (46%, compared to 70% in controls) and females (41%, compared to 71% in controls), largely attributable to the number of mice sacrificed "in extremis" following self-trauma. The observed depression in mean body weight and mean body weight gain was interpreted to be due to the interplay of increased incidence and severity of dermal self-trauma and mild systemic toxicity. Only animals from the ≤ 150 ppm groups were evaluated for carcinogenicity. No treatment-related tumours were reported.

In the **rat study**, rats were fed esfenvalerate at a concentration of 0, 15, 50, 150 or 400 ppm (~0.7, 2.3, 6.9, 18.5 mg/kg bw/d for males and 0.8, 2.7, 8.0 and 21.5 mg/kg bw/d for females). The top dose (400 ppm) was selected based on signs of toxicity including deaths seen at 500 ppm and above in repeated dose studies. There were no treatment-related clinical signs, or effects on survival rates. Body weights were reduced in treated males; the effect was statistically significant at the top dose only.

In the control and top dose groups, all animals were examined, while in the 15, 50 and 150 ppm groups, only the animals with gross lesions or those found dead were subject to histopathological investigation. This has to be taken into consideration when the percentages of tumours are compared in the different dose groups. Overall, concerning the benign and malignant tumours (except the Leydig cell tumours, discussed later) found in the study, in some cases the percentages of tumours in the control and 400 ppm groups are practically identical (e.g. the pituitary gland (adenoma pars anterior) in females), and/or the incidence of tumours in the lower dose groups is higher than in the high dose group (e.g. benign thymoma, females), leading to the conclusion that there is no dose response. In addition, the percentage of tumours in the high dose groups do not exceed the 5 year historical control range. RAC agrees with the DS that there is no evidence of carcinogenic activity in the pituitary, the haemolymphoreticular system, thymus or mammary gland.

Concerning the Leydig cell tumours found in the original rat study, the EFSA peer review of esfenvalerate (EFSA, 2014) suggested that a classification of Carcinogenicity Category 2 may be appropriate. The Applicant disagreed with this proposal and conducted a histopathological examination of the testes in all animals of the intermediate dose groups to clarify the total incidence of Leydig cell tumours, since only decedent animals from these groups were examined in the original study. This additional histopathological examination was not available for EFSA peer review before the renewal of the approval decision. The additional study did not reveal any new preneoplastic or neoplastic lesions. The revised % incidence of benign Leydig tumours was therefore 4%, 2%, 0%, 8% and 8%, and the incidence of Leydig cells hyperplasia was 2%, 1%, 0%, 1% and 0% in the control, 15, 50, 150 and 400 ppm groups respectively.

A comprehensive battery of **mechanistic studies** were conducted on esfenvalerate as part of the US EPA's 'Endocrine Disruptor Screening Program' (*Table 3*). The following tests were carried out:

- Rat 26-week dietary hormonal study in males: no treatment related effects were found on serum luteinizing hormone and testosterone.

- Rat 10-day Hershberger bioassay for detecting androgenic activity (OECD 441): there were no treatment related changes in endocrine / reproductive organ weights (androgenic or anti-androgenic activity).
- Rat pubertal development and thyroid function in intact juvenile / peripubertal males (U.S. EPA, OPPTS 890.1500): no treatment related effects were reported on pubertal development, on serum levels of T4, TSH or testosterone, or on endocrine / reproductive organ weights and histopathology.
- In vitro estrogen receptor transcriptional activation (OECD 455): no agonist activity.
- In vitro H295R steroidogenesis assay (OECD 456): no induction or inhibition of steroid biosynthesis was found up to the limit of solubility in this assay (no treatment related changes in hormone levels).
- In vitro aromatase inhibition using human recombinant microsomes (U.S. EPA, OPPTS 890.1200): No significant inhibition of aromatase activity was reported up to the limit of solubility.

All tests were negative, showing no evidence of endocrine disruptive/carcinogenic activity, although RAC notes that not all relevant mechanisms inducing Leydig cell tumours (luteinising hormone, prolactin and dopamine related modes of action) were investigated thoroughly.

Overall, the incidence of the Leydig cell tumours was above the control in the two top doses, but without statistical significance and with no clear dose response. The tumours were benign, they occurred in one species only, while there were no preneoplastic lesions (Leydig cells hyperplasia) above the control. Esfenvalerate showed no genotoxic potential in the in vitro and in vivo studies, and no endocrine disruptive effects in a battery of mechanistic studies. Taking into consideration the data above, RAC supports the DS's proposal **not to classify for carcinogenicity**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Esfenvalerate is an insecticidal active substance used for the control of pests in agriculture, horticulture, forestry and amenity use. At the time of submission, there were no registrations for this substance under REACH. Esfenvalerate has existing entry in Annex VI of CLP with a harmonised classification for environmental hazards as Aquatic Acute 1 and Aquatic Chronic 1 and a generic M-factor of 10000. The review is targeted towards the evaluation of the existing entry for aquatic toxicity due to the new data.

Some of the environmental studies were conducted using fenvalerate as the test substance. Fenvalerate [(αRS)-α-cyano-3-phenoxybenzyl (2RS)-2-(4-chlorophenyl)-3-methylbutyrate (CAS; 51630-58-1)] is a mixture of four optical isomers, one of which is esfenvalerate ((S)-α-cyano-3-phenoxybenzyl-(S)-2-(4-chlorophenyl)-3-methylbutyrate), present at approximately 23%. These studies have been included in cases where equivalent esfenvalerate data were not available at the time of earlier evaluations, however they are largely supporting information and none of the critical endpoints relate to fenvalerate.

Overall, the DS concluded that esfenvalerate is not rapidly degradable, potentially bioaccumulative, and proposed classification as:

Aquatic Acute 1 with an M-factor of 10000, based on lowest 96-hour LC_{50} value for fish (*Rainbow trout*) of 0.0001 mg/L; and

Aquatic Chronic 1 with an M-factor of 10000 based on lowest NOEC value for invertebrates (*Daphnia magna*) of 0.0000018 mg/L.

Degradation

In the preliminary (Tier 1) hydrolysis study (OECD TG 111, GLP), esfenvalerate at temperature 50°C was found to be hydrolytically stable at pH 4. At pH 7 and 9, >10% AR hydrolysis occurred after 5 days. Consequently a Tier 2 test was performed at pH 7 and 9. In the Tier II test, buffer solutions were incubated at pH 7 at 40, 50 and 60ºC and at pH 9 at 25, 40 and 50°C in the dark for up to 32 days. Two major hydrolytic degradants were CPIA and PBald. CPIA was observed at maximum levels in the range of 41.6% to 93.4% AR and PBald was observed at maximum levels of 36.0% to 90.9% AR. The incubations at higher temperatures at pH 7 (50 and 60°C) resulted in the degradant $CONH_2$-Fen exceeding 10% AR (max. level observed in range of 10.1 to 11.1% AR). Under alkaline conditions, CONH2-Fen further degraded to 3-phenoxymandelic acid (max. of 8.6% to 11.3% AR) and CPIA-carboxamide (max. of 10.2% to 12.2% AR) across all three temperature ranges. The DT_{50} values at pH 7 and pH 9 ranged from 3.3 to 427.7 days and 2.7 hours to 5.3 days, respectively (Graham and Gilbert, 2012).

According to one aquatic photolysis study (Graham and Dove, 2012, OECD TG 316, GLP), esfenvalerate was seen to degrade under photolytic conditions to <1.7% AR by 21 DAT. The major degradants observed were PBacid, Dec-fen A, Dec-fen B and PA-Fen, with the mean maximum levels >10% AR. The degradants Dec-fen A, Dec-fen B and PA-Fen all reached maximum levels at 7 DAT, before declining, while PB-acid reached maximum levels at 14 DAT, before declining slightly. The DT_{50} value for esfenvalerate under irradiated conditions, equivalent to UK/US summer sunlight, was 2.0 days. The dark controls showed no significant degradation of esfenvalerate. The results of a second study (Suzuki, Fujisawa and Katagi, 2012) were only calculation based using the results from the first study. The input data were obtained from the report on photodegradation and quantum yield in sterile, aqueous solution of esfenvalerate (Graham and Dove, 2012). The calculated photolytic half-lives of esfenvalerate were 1.28 to 1.36 days at latitudes of 30, 40 and 50ºN in summer.

In a ready biodegradation study following OECD TG 301B, esfenvalerate was considered not readily biodegradable as the theoretical yield of evolved CO_2 from esfenvalerate was 0% after 28 days (Graham and Fenley, 2011).

Regarding water/sediment studies, the degradation of esfenvalerate was investigated in two aquatic systems using one radiolabelled form of esfenvalerate (Lewis, 1995). In two water/sediment systems, esfenvalerate dissipated very rapidly from the water phase (first order DT_{50} 5.3 to 8.9 days). This was due to both partitioning into sediment and to degradation. For both aquatic systems after 100 days, water contained mainly CPIA (44% to 48% AR) with small amounts of esfenvalerate (2.7% to 3.4% AR) and sediments contained mainly esfenvalerate (26% to 27% AR) with only small amounts of CPIA (4.1% to 5.4% AR). Kinetic re-evaluation of the water sediment studies (Jarvis and Mamouni, 2011) was undertaken in accordance with FOCUS kinetic guidance. Overall, the results for degradation and fate in simulated water/sediment systems for up to 100 days where DT_{50} = 25.3 to 30.7 days (total system, normalised to 20°C). Mineralisation after 100 days = 3.2% to 5.2%. DT_{90} = 216.9 to 263.3 days (total system at 10°C). Esfenvalerate was observed to dissipate rapidly to the sediment layer and degrade in water-sediment systems to several degradation products.

Overall, due to the results summarised above, the DS concluded that esfenvalerate should be considered as not rapidly degradable, according to the CLP criteria.

Aquatic Bioaccumulation

A reliable experimental fish bioconcentration factor (BCF) for Common carp (*Cyprinus carpio*) was available (Anonymous, 1991). The level of [^{14}C-chlorophenyl] esfenvalerate reached a "plateau" and was relatively stable from 7 to 28 days of exposure. Residues of total radioactivity and esfenvalerate in the whole fish after 28 days of exposure were 161 and 110 µg/kg, respectively, and the corresponding bioconcentration factors (BCF) were 2850 and 3110. For [^{14}C-phenoxypheny] esfenvalerate, residues of total radioactivity and esfenvalerate in whole fish at the end of the exposure period were 225 and 168 µg/kg, respectively. The corresponding BCF values were 3340 and 3650. The depuration half-lives for total radioactivity and esfenvalerate were calculated to be 6.89 and 7.80 days for phenoxypheny-labelled material and 6.50 and 7.88 days for chlorophenyl-labelled material. The BCF of 3110 was used in EU RoA List of Endpoints. The DS also provided two valid log P_{ow} values of 6.24 at 25°C (pH not stated) and 5.0 at 23°C (pH 7.3). Both of them were determined following OECD TG 107 and are greater than the trigger value of ≥ 4. Log Pow of 6.24 was accepted by EU RoA evaluation.

Overall, due to the results summarized above DS concluded that esfenvalerate should be considered as having a high potential for bioaccumulation. However, the DS noted that since esfenvalerate is considered not rapidly degradable and adequate chronic data are available, this would not affect the decision on chronic classification and M-factor.

Aquatic Toxicity

The aquatic toxicity test results from available acute and chronic studies for all trophic levels of esfenvalerate are summarised in the following table and sections. Fish and aquatic invertebrates were the most sensitive trophic groups.

The available data indicate that all the metabolites for which data are available are much less toxic to fish, invertebrates and algae than the parent esfenvalerate (by at least two orders of magnitude). Therefore, the DS did not consider them further for the classification of esfenvalerate.

Table: *Aquatic Toxicity results*

Test organism / guideline, test method	Short-term result (endpoint)	Long-term result (endpoint)	Test material	Reference
Fish				
Bluegill sunfish *(Lepomis macrochirus)* / Not specified, not GLP	96h LC_{50} = 0.00021 mg/L (nominal concentration)		Esfenvalerate, purity not specified	Anonymous (1985k)
Rainbow trout *(Salmo gairdneri)* / EPA 660/3-75-009, GLP	96h LC_{50} = 0.00026 mg/L (nominal concentration)		Esfenvalerate, (98.8%)	Anonymous (1985l)
Rainbow trout *(Salmo gairdneri)* / OECD TG 203, not GLP	96h LC_{50} = 0.0001 mg/L (nominal concentration)		Esfenvalerate, (94.5%)	Anonymous (1986f)
Fathead Minnow *(Pimephales promelas)* / EPA 660/3-75-009, not GLP	96h LC_{50} = 0.00018 mg/L (nominal concentration)		Esfenvalerate, (98.8%)	Anonymous (1984b)
Rainbow trout *(Salmo gairdneri)* / OECD TG 204*, GLP Prolonged fish toxicity test		21d NOEC = 0.000001 mg/L (mean measured)	Esfenvalerate, (97%)	Anonymous (1991b)

Test organism / guideline, test method	Short-term result (endpoint)	Long-term result (endpoint)	Test material	Reference
Fathead Minnow *(Pimephales promelas)* / US EPA "Recommended bioassay procedure for fathead minnows", not GLP		260d NOEC = 0.00009 mg/L (mean measured)	Fenvalerate, (96%) includes esfenvalerate isomers / full life cycle	Anonymous (1978b)
Aquatic invertebrates				
Water flea (*Daphnia magna*) / OECD TG 202, GLP	48h EC_{50} = 0.027 mg/L (mean measured)		Esfenvalerate, (86.6%)	Sayers (2005)
Water flea (*Daphnia magna*) / US EPA 72-2, GLP	48h EC_{50} = 0.0009 mg/L (nominal concentration)		Esfenvalerate, (98.6%)	Hutton (1987)
Water flea (*Daphnia magna*) / US EPA 72-2, GLP	48h EC_{50} = 0.0035 mg/L (nominal concentration)		Esfenvalerate, (98.6%)	Hutton (1987)
Water flea (*Daphnia magna*) / OECD TG 202, GLP	48h EC_{50} = 0.00021 mg/L (mean measured) 48h EC_{50} ~ 0.000045 mg/L (mean measured)		Esfenvalerate Aβ isomer, (98.8%) Esfenvalerate (87.3%)	Sayers (2011)
Water flea (*Daphnia magna*) / US EPA 72-4, GLP		21d NOEC = 0.000052 mg/L (mean measured)	Esfenvalerate, (98.6%)	Hutton (1987)
Water flea (*Daphnia magna*) / OECD TG 202, GLP		21d NOEC = 0.0000018 mg/L (nominal concentration)	Esfenvalerate, (97%)	Handley *et al*. (1991)
Algae				
Green algae *(Scenedesmus subspicatus)* / OECD TG 201, GLP	96h E_bC_{50} = 0.0065 mg/L 24-48-h E_rC_{50} = 0.01 mg/L (nominal concentration)	96h NOEC = 0.001 mg/L (nominal concentration)	Esfenvalerate, (97%)	Handley *et al*. (1991)
Other aquatic organisms				
Non-biting midge *(Chironomus riparius)* / Guideline BBA, GLP		28d NOEC = 0.00016 mg/L (nominal concentration)	Esfenvalerate, (98.9 %)	Putt (1997)

*The prolonged fish toxicity test guideline (OECD TG 204) is not considered a chronic test according to ECHAs CLP guidance and has been deleted by the OECD. Therefore, Anonymous (1991b) was included only as supporting information by the DS.

Acute Aquatic Toxicity

Four studies were submitted on the acute toxicity of esfenvalerate in fish. Rainbow trout were identified as the most acutely sensitive fish species by the DS. The study was performed according to the OECD TG 203 and the resulting LC_{50} value of 0.0001 mg/L was the lowest among

of the four studies. However this endpoint was based on nominal concentrations; measured concentrations were 107-125% of nominals and so close to, but exceeding, 80-120%. An LC50 based on measured concentrations was not presented but as the measured concentrations were 107-125% of nominal, would be slightly above 0.0001 mg/L. This result should therefore be treated with some caution but it was considered acceptable for hazard classification purposes by the DS.

Four studies were submitted on the acute toxicity of esfenvalerate in invertebrates (*Daphnia magna*). The reported 48h EC_{50} values for *Daphnia magna* were 0.027, 0.0009, 0.0035 and <0.000049 mg/L (however, as 55% immobilisation was seen at this last concentration, the actual EC_{50} was assumed to be approximately 0.000045 mg/L).

In addition to this last study (Sayers, 2011), an EC_{50} value of 0.00021 mg/L resulting from testing *Daphnia magna* with esfenvalerate 2SαR-isomer (Aβ isomer) was also provided by the same study author. This comparative study gave the lowest endpoint for technical esfenvalerate under similar test conditions, indicating that it was more toxic than the 2SαR-isomer.

As the four endpoints endpoints for technical esfenvalerate (i.e. 0.027, 0.0009, 0.0035 and ~0.000045 mg/L) appear to have been derived under the same conditions, the DS opted to calculate a geometric mean value for *Daphnia magna*. This was done according to the ECHA Guidance on the Application of the CLP Criteria (2017). This results in a geomean value for the acute toxicity to aquatic invertebrates of 0.0014 mg/L.

A study submitted on the toxicity of esfenvalerate to green algae (*Scenedesmus subspicatus*) indicated 96h E_bC_{50} of 0.0065 mg/L and E_rC_{50} value of 0.01 mg/L based on nominal concentrations.

Overall, the DS proposed classify esfenvalerate as Aquatic Acute category 1 based on the 96h LC_{50} for rainbow trout of 0.0001 mg/L. As this acute toxicity value falls within the $0.00001 < L(E)C_{50} \leq 0.0001$ mg/L range, the acute M-factor proposed by the DS is 10000.

Aquatic Chronic Toxicity

Two studies were submitted on the chronic toxicity of esfenvalerate in fish. One of them was carried out with rainbow trout, which was the most sensitive species under acute testing. However, it was undertaken following the now deleted OECD TG 204 (prolonged juvenile fish growth test guideline). The NOEC value of 0.000001 mg/L (based on nominal concentrations) derived from this study was the lowest. The other chronic fish study was a full fish life-cycle test carried out with fathead minnow and fenvalerate (96%) following US EPA guideline (1971), which includes esfenvalerate isomers. The 260d NOEC from this study was 0.00009 mg/L.

Two studies were submitted on the chronic toxicity of esfenvalerate in *Daphnia magna*. The lowest NOEC value was 0.0000018 mg/L after 21 days. This endpoint was based on nominal concentrations and there was no clear confirmation of measured concentrations in actual test media. However, as it represents the lowest chronic NOEC value amongst all aquatic invertebrate tests, it was considered for classification purposes by the DS.

A study were submitted on the toxicity of esfenvalerate to green algae (*Scenedesmus subspicatus*) indicating a 96h NOEC of 0.001 mg/L (based on nominal concentration).

A study on the chronic toxicity of esfenvalerate to non-biting midge (*Chironomus riparius*) was submitted as well. The resulting 28d NOEC value was determined to be 0.00016 mg/L (emergence), based on initial nominal concentrations in the water phase. However, as mean measured endpoints in the water phase have not been determined, these results are of uncertain reliability for hazard classification.

In addition, the DS noted two reliable GLP studies on the endocrine disrupting (ED) potential as suitable for chronic aquatic hazard classification purposes. The first of these was a flow-through

Fish Short-Term Reproduction Assay (to OPPTS 890.1350 and EPA 740-C-09-007 guidelines) on fathead minnow (*Pimephales promelas*) by Anonymous (2012d). The overall measured NOEC was therefore 0.000231 mg a.s./L, the highest concentration tested. The second ED study was a 21 day amphibian metamorphosis assay (to U.S. EPA and OPPTS guideline 890.1100 (2009)) by D. J. Fort (2012). The overall NOEC for esfenvalerate was 0.0000397 mg a.s./L (the highest concentration tested). However, as these values indicate lower toxicity than for other species, the DS did not consider them important for the chronic hazard classification proposal.

Overall, the DS proposed to classify esfenvalerate as Aquatic Chronic category 1 based on the 21d NOEC for *Daphnia magna* of 0.0000018 mg/L. As the substance is 'not rapidly degradable', as well as potentially bioaccumulative and chronic toxicity value falls within the 0.000001 < L(E)C_{50} ≤ 0.00001 mg/L range, the chronic M-factor proposed by the DS is 10000.

Comments received during public consultation

Four Member States (MSs) submitted comments. All commenting MSs agreed with the proposed classification and M-factors. However, two of them did not agree to use the geomean method for acute toxicity in *daphnia*. They both indicated that the 48h EC_{50} of 0.0035 mg/L for *Daphnia magna* is not reliable because the daphnids were fed during the study. According to OECD TG 202, the daphnids should not be fed during the test. Also, the 48h EC_{50}s of 0.0009 and 0.0035 mg/L are derived from unreliable studies, since in both tests the test compound was not measured during the test. It was argued that due to those reasons, these results cannot be compared to the other EC_{50} results for *Daphnia magna* and there are not enough data to calculate the geometric mean (ECHA CLP-guidance, 2017). The lowest EC_{50} value of *Daphnia magna* for aquatic acute classification would be 0.000045 mg/L. However, it would not change the classification from the original proposals. In response, the DS agreed that feeding the daphnia could have affected the results in the study along with the stability of the test substance, which was not determined during the test. For both reasons, the DS agreed that these endpoints (48h EC_{50} of 0.0009 and 0.0035 mg/L) are potentially unreliable and the geomean calculation for Daphnia is not appropriate.

The DS agreed that the lowest acute EC_{50} value is actually the EC_{50} of 0.000045 mg/L for invertebrates rather than the fish LC_{50} of 0.0001 mg/L for fish. However, they mentioned that both values are in the same range >0.00001 to ≤ 0.0001 and, therefore, both values support the proposed classification of Aquatic Acute 1 with an Acute M-factor of 10000.

Assessment and comparison with the classification criteria

Degradation

Esfenvalerate is hydrolytically stable under acidic conditions (pH 4, 50 °C), but undergoes hydrolysis under neutral and alkaline conditions across all temperature ranges (>10 % degradation after 5 days). DT_{50} values at pH 7 and at pH 9 were ranged from 3.3 to 427.7 days and from 2.7 hours to 5.3 days, respectively, at 20°C. It undergoes rapid aqueous photolytic degradation to primary degradants but toxicity information on all primary degradation products is not available. Esfenvalerate is also of low volatility. Esfenvalerate dissipated rapidly from the water phase to the sediment. However the whole system half-life in a natural water-sediment system ranged from 25.3 to 30.7 days.

A ready biodegradation study with esfenvalerate indicated 0% degradation after 28 days, indicating that esfenvalerate is not readily biodegradable.

Consequently, RAC agrees with the DS that esfenvalerate should be considered not rapidly degradable for the purpose of classification under CLP.

Aquatic Bioaccumulation

The representative experimental whole fish BCF of 3110 of esfenvalerate is substantially above than the CLP BCF threshold of 500. Although there was no normalisation for lipid content or growth, RAC considers that this would not substantially alter the results and would not influence the conclusion regarding bioaccumulation potential. Available log Pow values of 6.24 at 25°C (pH not stated) and 5.0 at 23°C (pH 7.3) are also above the CLP criterion of ≥ 4. Therefore, RAC agrees with the DS that esfenvalerate is bioaccumulative according to the CLP criteria.

Aquatic Toxicity

RAC notes that there are reliable acute and chronic aquatic toxicity data for fish, aquatic invertebrates and algae. RAC agrees that, based on available data provided by DS, the parent substance (esfenvalerate) is more toxic than the degradation products. The most acutely and chronically sensitive species were invertebrates (*Daphnia magna*) and fish (Rainbow trout). The identified main degradants are less acutely toxic than the parent substance and therefore they are not considered further in relation to the classification of esfenvalerate.

Aquatic Acute

RAC concludes that the geometric mean of toxicity values of *Daphnia magna* should not be used as an aquatic acute toxicity value for that species. 48h EC_{50}s of 0.0009 and 0.0035 mg/L values (Hutton D. G., 1987) were derived from unreliable studies since in both tests the test compound was not measured during the test. Also, the 48h EC_{50} of 0.0035 mg/L for *Daphnia magna* is not reliable because the daphnids were fed during the study. As these aquatic acute toxicity values should be excluded, the geometric mean approach cannot be applied and aquatic acute classification should be based on the lowest reliable toxicity value, a 48h EC_{50} of 0.000045 mg/L for *Daphnia magna*.

Overall, RAC agrees with the DS's proposed classification as Aquatic Acute 1 (M-factor 10000) based on a 48h EC_{50} of 0.000045 mg/L value for *Daphnia magna*.

Aquatic Chronic

RAC did not use for the aquatic chronic classification purposes the OECD TG 204 test results 21d NOEC of 0.000001 mg/L with *Rainbow trout* (Anonymous, 1991b). However, RAC acknowledged that rainbow trout could potentially be slightly more sensitive than *Daphnia magna* for aquatic chronic toxicity.

Overall, RAC agrees with the proposed classification by the DS as Aquatic Chronic 1 (M-factor 10000) and confirms that the lowest chronic/long-term endpoint value for aquatic chronic classification purpose of esfenvalerate is the 21d NOEC value for *Daphnia magna* of 0.0000018 mg/L.

Conclusion on classification

Esfenvalerate is considered as not rapidly degradable and bioaccumulative according to the CLP criteria. Based on the available and reliable information, RAC concludes that esfenvalerate warrants classification as:

Aquatic Acute 1 based on 48-hours EC_{50} of 0.000045 mg/L for *Daphnia magna*. As this chronic toxicity value falls within the 0.00001 < L(E)C50 ≤ 0.0001 mg/L range, the **acute M-factor is 10000.**

Aquatic Chronic 1 based on 21-d NOEC of 0.0000018 mg/L for *Daphnia magna*. As this chronic toxicity value falls within the 0.000001 < NOEC ≤ 0.00001 mg/L range, the **chronic M-factor is 10000.**

Additional references

Bradberry, S.M., Cage, S.A., Proudfoot, A.T. et al. Poisoning due to pyrethroids. Toxicol Rev (2005) 24: 93. https://doi.org/10.2165/00139709-200524020-00003

Wilkes MF. Pyrethroid-induced paresthesia: a central or local toxic effect? J Toxicol Clin Toxicol 2000; 38: 103-5. https://www.ncbi.nlm.nih.gov/pubmed/10778905

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).