Committee for Risk Assessment

RAC

Annex 1

Background document

to the 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

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

Adopted

20 September 2019

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

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

EC Number: **-**

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

Index Number: **608-058-00-4**

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Chemicals Regulation Division
Health and Safety Executive
United Kingdom

Version number: 2 **Date: October 2018**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON ESFENVALERATE (ISO); (*S*)-α-CYANO-3-PHENOXYBENZYL-(*S*)-2-(4-CHLOROPHENYL)-3-METHYLBUTYRATE

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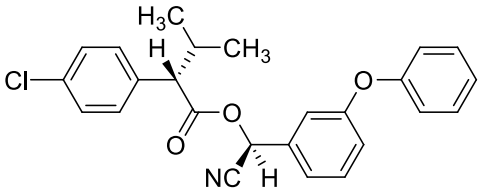
ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON ESFENVALERATE (ISO); (*S*)-α-CYANO-3-PHENOXYBENZYL-(*S*)-2-(4-CHLOROPHENYL)-3-METHYLBUTYRATE

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	(*S*)-α-cyano-3-phenoxybenzyl-(*S*)-2-(4-chlorophenyl)-3-methylbutyrate
Other names (usual name, trade name, abbreviation)	Esfenvalerate
ISO common name (if available and appropriate)	Esfenvalerate
EC number (if available and appropriate)	Not assigned
EC name (if available and appropriate)	Not assigned
CAS number (if available)	66230-04-4
Other identity code (if available)	Not applicable
Molecular formula	$C_{25}H_{22}ClNO_3$
Structural formula	
SMILES notation (if available)	Not applicable
Molecular weight or molecular weight range	419.91 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	83% of SS isomer
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable.
Minimum purity of the active substance as manufactured **Degree of purity (%) (if relevant for the entry in Annex VI)**	830 g/kg

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
Esfenvalerate	≥83%	Acute Tox 3*; H301 Acute Tox 3*; H331 Skin Sens 1; H317 Aquatic Acute 1; H400 Aquatic Chronic 1; H410	Acute Tox 3; H301 Acute Tox 3; H331 Skin Sens 1; H317 Aquatic Acute 1; H400 Aquatic Chronic 1; H410

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
Toluene	1% maximum	Skin Irrit. 2: H315 Asp. Tox. 1: H304 STOT SE 3: H336 STOT RE 2: H373 Repr. 2: H316d	N/A	No
Sum of Aβ + Bα + Bβ isomers	Confidential	N/A	N/A	Yes

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Not applicable

Table 5: Test substances (non-confidential information) (this table is optional)

Not required

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

Proposed harmonised classification and labelling according to the CLP criteria

2.1

Table 6:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	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	GHS09 GHS06 Dgr 	H301 H331 H317 H410		M(Chronic) = 10000	
Dossier submitters proposal	608-058-00-4	esfenvalerate (ISO); (*S*)-α-cyano-3-phenoxybenzyl-(*S*)-2-(4-chlorophenyl)-3-methylbutyrate	-	66230-04-4	**Modify** Acute Tox 3 **Modify** Acute Tox 2 **Retain** Skin Sens 1 **Add** STOT RE Cat 2 **Retain** Aquatic Acute 1 **Retain** Aquatic Chronic 1	H301 H330 H317 H373 H400 H410	GHS09 GHS06 GHS08 Dgr	H301 H330 H317 H373 H410		**Add** ATE_{oral} = 88.5 mg/kg **Add** ATE_{inhal} = 0.48mg/L **Add** M(Acute) = 10000 **Retain** M(Chronic) = 10000	

Resulting Annex VI entry if agreed by RAC and 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 2 Skin Sens 1 STOT RE Cat 2 Aquatic Acute 1 Aquatic Chronic 1	H301 H330 H317 H373 H400 H410	GHS09 GHS06 GHS08 Dgr	H301 H330 H317 H373 H410		ATE_{oral} = 88.5 mg/kg ATE_{inhal} = 0.48mg/L M(Acute) = 10000 M(Chronic) = 10000	

* minimum classification in the translation from classification under Directive 67/548/EEC (DPD) to classification under the CLP regulation

Table 7: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	Hazard class not assessed in this dossier	No
Oxidising gases	Hazard class not assessed in this dossier	No
Gases under pressure	Hazard class not assessed in this dossier	No
Flammable liquids	Hazard class not assessed in this dossier	No
Flammable solids	Hazard class not assessed in this dossier	No
Self-reactive substances	Hazard class not assessed in this dossier	No
Pyrophoric liquids	Hazard class not assessed in this dossier	No
Pyrophoric solids	Hazard class not assessed in this dossier	No
Self-heating substances	Hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	Hazard class not assessed in this dossier	No
Oxidising liquids	Hazard class not assessed in this dossier	No
Oxidising solids	Hazard class not assessed in this dossier	No
Organic peroxides	Hazard class not assessed in this dossier	No
Corrosive to metals	Hazard class not assessed in this dossier	No
Acute toxicity via oral route	**Harmonised classification proposed**	**Yes**
Acute toxicity via dermal route	Hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	**Harmonised classification proposed**	**Yes**
Skin corrosion/irritation	Hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	Hazard class not assessed in this dossier	No
Respiratory sensitisation	Hazard class not assessed in this dossier	No
Skin sensitisation	**Harmonised classification proposed**	**Yes**
Germ cell mutagenicity	**Data conclusive but not sufficient for classification**	**Yes**
Carcinogenicity	**Data conclusive but not sufficient for classification**	**Yes**
Reproductive toxicity	Hazard class not assessed in this dossier	No
Specific target organ toxicity-single exposure	**Data conclusive but not sufficient for classification**	**Yes**
Specific target organ toxicity-repeated exposure	**Harmonised classification proposed**	**Yes**
Aspiration hazard	Hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	**Harmonised classification proposed**	**Yes**
Hazardous to the ozone layer	Hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Esfenvalerate was included in Annex I of Directive 67/548/EEC with a classification of T; R23/25, R43 and N; R50-53. The classification was subsequently "translated" to give the following classification in Annex VI of the CLP Regulation (EC) 1272/2008): Acute Tox 3* (H301), Acute Tox 3* (H331), Skin Sens 1 (H317), Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410) (the '*' indicates that this is a minimum classification).

At the time of submission, esfenvalerate is not registered under REACH (Regulation (EC) 1907/2006).

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Esfenvalerate is a pesticide active substance. As a result of the renewal assessment under Regulation EC 1107/2009, it is appropriate to review the existing entry in Annex VI of CLP. This proposal is targeted towards:

Acute oral and inhalation toxicity and skin sensitisation: change in existing Annex VI entry due to changes in the classification criteria (i.e., change from Directive 67/548/EEC to EC 1272/2008, and introduction of subcategorisation of skin sensitisers with the 2nd ATP to CLP).

Carcinogenicity: new data are available. In the original DAR, data on carcinogenicity were not available for esfenvalerate (instead, data on fenvalerate were used for this endpoint). For the renewal review, a carcinogenicity study in rats on esfenvalerate was provided by the applicants. An 18 month study using esfenvalerate in mice is also available. During the pesticide review process, a concern for carcinogenicity was raised in the EFSA conclusion. This was based on the incidence of testicular interstitial (Leydig) cell tumours observed in the two-year chronic toxicity/carcinogenicity dietary study with esfenvalerate in Wistar rats. Therefore, data on carcinogenicity are considered in this proposal to assess whether classification for carcinogenicity is required.

Repeated dose toxicity: new data are available.

Aquatic toxicity: evaluation of existing entry due to new data

This CLH report considers the following endpoints: acute toxicity (oral and inhalation routes), STOT SE, skin sensitisation, carcinogenicity and STOT RE. Consideration is also given to data on mutagenicity, as this will aid the assessment of carcinogenicity.

5 IDENTIFIED USES

Esfenvalerate is a broad spectrum contact and ingested pyrethroid insecticide used for the control of pests in agriculture, horticulture, forestry and amenity use. It is especially effective against Coleoptera, Diptera, Hemiptera, Lepidoptera and Orthoptera.

6 DATA SOURCES

Esfenvalerate was evaluated for renewal of approval as a pesticide active substance according to Commission Regulation (EU) No 1141/2010. The primary sources of data are:

1. The company reports and published data contained in the renewal of approval dossier submitted by the applicant (Sumitomo Chemical Company)
2. The Renewal Assessment Report (RAR) plus associated documentation published by EFSA, as follows:

   EFSA RAR Volumes 1-4 (2014)

   EFSA LoEP (2014)

   EFSA Conclusion (2014)

It should be noted that some of the studies included in this report 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%.

7 PHYSICOCHEMICAL PROPERTIES

A summary of the physicochemical properties of esfenvalerate is provided to aid the evaluation of toxicity hazards for human health and the environment (aquatic toxicity). The information is extracted from the RAR, Esfenvalerate Volume 3 – Annex B.2: Physical and chemical properties, June 2014 (refer to Annex 1).

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at room temperature	White, crystalline solid (pure substance) Yellow, viscous liquid (technical material)	Furuta R. (1995a); LLP-0051; RAR B.2.1.7, B.2.1.8 Furuta R. (1995b); LLP-0057; RAR B.2.1.7, B.2.1.8	Visual inspection Purity: 99.4% (pure), 85.3% (technical material)
Melting/freezing point	59.1 to 60.1°C	Russel S. (1995); LLP-0059; RAR B.2.1.1	EEC method A.1 Purity: 99.9%
Boiling point	Boiling point: 355.97°C. A red brown glassy solid remained in the crucible after the test suggesting the material had undergone decomposition.	Leslie S., Moseley R.H. (2011); LLP-0100; RAR B.2.1.2	EEC method A 2, OECD 103 Purity: 100%
Relative density	1.2338 at 20.2°C (Density = 1.2338 g/cm^3 at 20.2 °C)	Leslie S., Moseley R.H. (2011); LLP-0100; RAR B.2.1.4	EEC method A 3, OECD 109 Purity: 100%
Vapour pressure	1.17 x 10^{-9} Pa at 20°C 2.84 x 10^{-9} Pa at 25°C (extrapolated from the log of the experimentally determined vapour pressure versus 1/temperature plot)	Wells D.F. (1998); LLP-0074; RAR B.2.1.5	EEC Method A.4 OECD 104 (gas saturation method) Purity: 99%
Surface tension	Water solubility is too low to require determination of surface tension according to EEC A.5	Russell S. (1995); LLP-0059; RAR B.2.1.24	EEC method A.5 Purity: not relevant (theoretical evaluation)

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Water solubility	<1µg/L at 20°C (Test conditions measured at pH 5.4 No effect of pH expected due to no dissociation)	Kogovsek L.M. (1997); LLP-0066: RAR B.2.1.11	EEC method A.6 (shake flask method) Purity: 100%
Volatility, Henry's law constant	4.92 x 10^{-4} Pa m^3 mol^{-1} at 20°C	Yoshimura J. (1998); LLP-0078; RAR B.2.1.6	Not applicable, calculation only
Partition coefficient n-octanol/water	Log Pow = 6.24 at 25°C (pH not stated) Log P_{OW} = 5.0 at 23ºC (pH 7.3)	Tanoue A., Itoh K. (1989); LLP-0033; RAR B.2.1.13 Rohr G. (1991); LLP-0043; RAR B.2.1.13	OECD 107 Purity: 99.4% OECD 107 Purity 99.9%
Flash point	Flash point determined at: 241.4°C	Russell S. (1995); LLP-0059; RAR B.2.1.21	EEC method A.9 Purity: 97.8%
Flammability	Experience gained through handling and use, and analysis of the chemical structure, indicate that the substance is not pyrophoric and does not emit flammable gases upon contact with water	-	A10 study not conducted as submitted test material (esfenvalerate TGAI) was a liquid
Explosive properties	Not classified as explosive	Russell S. (1995); LLP-0059; RAR B.2.1.22	EEC Method A.14 Purity: 97.8%
Self-ignition temperature	Auto-ignition temperature determined at: 435 ± 5°C	Russell S. (1995); LLP-0059; RAR B.2.1.20	EEC Method A.15 Purity: 97.8%
Oxidising properties	Esfenvalerate is not considered capable of possessing oxidising properties; whilst it contains oxygen and chlorine atoms, these are bonded to carbon only. The calculated oxygen balance is -219%. This value is outside the region where there may be a potential for the test substance to be considered an oxidiser.	Leslie S., Moseley R.H. (2011); LLP-0100; RAR B.2.1.23	EEC Method A.17 Purity: not relevant (theoretical evaluation)
Granulometry	No data	-	Not relevant

Property	Value	Reference	Comment (e.g. measured or estimated)
Solubility in organic solvents	n-Heptane: 14-20 g/L 1,2-Dichloroethane: >250 g/L Methanol: > 50 g/L Acetone: >250 g/L p-Xylene: >250 g/L Ethyl acetate: >250 g/L	Leslie S., Moseley R.H. (2011); LLP-0100; RAR B.2.1.12	CIPAC Method MT 181 Purity: 87.3%
Dissociation constant	Data from UV spectra testing at different pH was evaluated to conclude that esfenvalerate does not exhibit a dissociation constant within the normal pH range. This is consistent with the structure of esfenvalerate which indicates no groups with appreciable acid or basic character.	Leslie S., Moseley R.H. (2011); LLP-0100; RAR B.2.1.18	OECD Test Guideline 112 Purity: 100%
Viscosity	No data	-	Not relevant

8 EVALUATION OF PHYSICAL HAZARDS

Not evaluated in this report.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

A summary of the toxicokinetic (ADME) data pertaining to esfenvalerate is provided to aid the evaluation of toxicity hazards for human health.

The study summaries are presented in Annex 1 and are taken from the RAR, Esfenvalerate - Volume 3 Annex B.6: Toxicology and metabolism, June 2014.

Some of the toxicokinetic studies use fenvalerate as a test substance. Fenvalerate is a pesticide, and is a mixture of four optical isomers which have different insecticidal activities. Fenvalerate consists of approximately 23% of the esfenvalerate isomer.

Table 9: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
Similar to OECD TG 417. Balance/WBA study in male rats, single and repeat dosing. Dose levels: Single dose: 8.4 mg/kg bw Repeated dose: 5 x 1.7 mg/kg bw/day	Rapidly excreted and low tissue levels with route, rate and highest tissue levels dependent on radiolabel. Maximum concentration of fenvalerate in blood reached within 3 hr after dosing and rapidly declined. Major metabolic pathways were oxidation at the 2- and 4- positions of the acid, and at the 2'- and 4'- positions of the alcohol, cleavage of the ester and conversion of the cyano group to SCN^- and $CO_{2.+}$	Test substances: [^{14}C-CN]-fenvalerate, [^{14}C-CN]-esfenvalerate, [^{14}C-carbonyl]-esfenvalerate, [^{14}C-benzylic]-esfenvalerate, [^{14}C-chlorophenyl]-chlorophenyl-isovaleric acid (CPIA) Vehicle: suspension in 10% tween 80	Ohkawa, H., *et al*. (1979) RAR B.6.1.1
Similar to OECD TG 417. Balance/WBA study in male and female rats and mice using single and pre-feeding doses. Dose levels: Single: fenvalerate: 7-8 and 30 mg/kg bw esfenvalerate: 4-5 mg/kg bw Pre-feeding: fenvalerate at 500 mg/kg bw in diet for 2 weeks followed by [^{14}C] fenvalerate 2.1 mg/kg bw (rats) and 8.4 mg/kg bw (mice)	Acute toxic symptoms in high dose rats 1-4 hours after dosing. Rapidly excreted and low tissue levels with route, rate and highest tissue levels dependent on radiolabel but independent of dose level. Major metabolic pathways were oxidation at the 2- and 4- positions of the acid, and at the 2'- and 4'- positions of the alcohol, cleavage of the ester and conversion of the cyano group to SCN^- and $CO_{2.}$	Test substances: fenvalerate [^{14}C-carbonyl], [^{14}C-benzylic] and [^{14}C-CN] labelled, esfenvalerate [^{14}C-chlorophenyl], [^{14}C-phenyl ring] and [^{14}C-CN] labelled Vehicle: suspension in 10% tween 80	Kaneko, H., *et al.* (1981) RAR B.6.1.2
Similar to OECD TG 417. Balance/WBA study in male and female rats and mice using single and repeat doses. Dose levels: Single: esfenvalerate 2.5 mg/kg bw (rats and mice) fenvalerate 2.5 and 10 mg/kg bw (rats and mice) Repeat: esfenvalerate 10 x 2.5 mg/kg bw/day (mice) Fenvalerate 10 x 10 mg/kg bw/day (mice)	Rapidly excreted and low tissue levels independent of dose level and species. Highest tissue residues were detected in fat. Major metabolic pathways were oxidation at the 2- and 4- positions of the acid, and at the 2'- and 4'- positions of the alcohol moiety, cleavage of the ester	Test substances: fenvalerate [^{14}C-phenoxybenzyl] and [^{14}C-chlorophenyl] labelled, esfenvalerate [^{14}C-phenoxybenzyl] and [^{14}C-chlorophenyl] labelled Vehicle: solution in corn oil	Anonymous (1985a) RAR B.6.1.3
Tissue depletion in male and female rats and mice following 28 days' dietary dosing with single dose level with and without a 28 day [^{14}C] washout period. Dose level: 25 ppm	The concentration of radioactivity in all tissues approached a plateau after 28 days administration and then declined during administration of untreated diet. Highest tissue residues were present in the fat.	Test substances: fenvalerate [^{14}C-chlorophenyl] labelled, esfenvalerate [^{14}C-chlorophenyl] labelled Vehicle: in diet, dissolved in corn oil	Anonymous (1985b) RAR B.6.1.4

<table>
<tr><th>Method</th><th>Results</th><th>Remarks</th><th>Reference</th></tr>
<tr><td>Similar to OECD TG 417. Balance study in bile duct cannulated and intact male rats. Cannulated rats received bile infusion from donor animals. Single oral dose, two dose vehicles compared. Dose level: 0.5 mg/kg bw</td><td>The excretion of radioactivity was higher in urine and lower in faeces for intact rats than in cannulated animals. Urinary excretion was slightly higher in animals dosed with 10% tween 80 than corn oil.</td><td>Test substance: esfenvalerate [^{14}C-phenoxyphenyl] labelled

Vehicles: corn oil or 10% tween 80</td><td>Anonymous (1998)

RAR B.6.1.5</td></tr>
<tr><td>Placental transfer of esfenvalerate to 13-day old pregnant rats following either a single dose or 3 consective daily doses. Before the 3rd oral dose, unlabelled compound was given to the animals. Total radioactivity in maternal blood, foetus, placenta, amniotic fluid and ovary was determined 3, 6, 12, 24 and 48 hours after single dosing, and 3, 6, 12, 24 and 48 hours after the last treatment of the repeated dosing
Dose levels: 2.5, 10 mg/kg bw/d</td><td>The placental transfer of esfenvalerate was investigated in pregnant rats following single or repeat dosing during gestation. Less than 0.07% of the applied radioactivity was found in the foetuses indicating that transfer of radioactivity from maternal blood to the foetuses did not readily occur. There was no evidence of accumulation of esfenvalerate in the foetal tissue or amniotic fluid of rats.</td><td>Test substance: Esfenvalerate with [^{14}C-chlorophenyl]label

Vehicle: corn oil</td><td>Anonymous (1985c)

RAR B.6.1.6</td></tr>
<tr><td>Metabolism study in dogs following a single oral (via gelatin capsule) dose. Urine, faeces and blood were collected daily for 3 days and metabolites were analysed using TLC. Dose level: 1.7 mg/kg bw</td><td>Fenvalerate was rapidly eliminated after administration. The recovery in urine and faeces for the two labelling positions is given below.
<table>
<tr><td></td><td colspan="2">^{14}C-label</td></tr>
<tr><td></td><td>Chloro-phenyl</td><td>Phenoxy-benzyl</td></tr>
<tr><td>Urine</td><td>31.6</td><td>36.8</td></tr>
<tr><td>Faeces</td><td>55.5</td><td>42.3</td></tr>
<tr><td>DT_{50} (d)</td><td>1.0</td><td>0.7</td></tr>
</table>
The biological half-life for fenvalerate in the blood was about 2 hr and the level of a.i. decreased below the detection limit (0.01 ppm) in 48 hr after dose.
Fenvalerate was metabolised mainly by oxidation at the 4’phenoxy position of the alcohol moiety and at the C-2 and C-3 positions of the acid moiety, cleavage of the ester linkage and conjugation of the resultant carboxylic acids, phenols and alcohols with glucuronic acid, sulphate and/or amino acid</td><td>Test substance: Fenvalerate [^{14}C-chlorophenyl] and [^{14}C-phenoxybenzyl]-labelled

Vehicle: gelatin capsule</td><td>Kaneko et al (1984)

RAR B.6.1.7</td></tr>
</table>

Method	Results	Remarks	Reference
The amount of cholesteryl [2R]-2-(4-chlorophenyl) isovalerate (CIPA-cholesterol ester) produced from specific isomers of fenvalerate was determined in tissues from rats and mice given a single oral gavage dose or mice given repeated dietary dose of radiolabelled various isomers of fenvalerate. Dose level: single dose 2.5 mg/kg bw (rats and mice), 70 mg/kg bw (male mice) Repeated dose: 7 or 14 days at 500 ppm in diet (male mice)	CPIA-cholesterol ester was found predominantly in mice and the [2R,αS]-isomer of fenvalerate was established as the only source of the CPIA-cholesterol ester.	Test substances: fenvalerate [^{14}C-chlorophenyl] and [^{14}C phenoxybenzyl] labelled Vehicle: suspension in 10% tween 80 or in diet, dissolved in corn oil	Anonymous. (1986) RAR B.6.1.8
The influence of isomeric form and animal species on production of CPIA - cholesterol ester from fenvalerate was investigated using microsomes from various tissues.	Mouse kidney, brain and spleen produced the most CPIA-cholesterol ester. Free CPIA was not a substrate for the formation of CPIA-cholesterol ester. CPIA-cholesterol ester was formed only from the [2R,αS]-isomer in all tissues and species examined, apart from the mouse kidney, which produced only a trace amount from the [2R,αR]-isomer	Test substances: Four [^{14}C-chlorophenyl] chiral isomers of fenvalerate ([2S, αS]; [2S, αR], [2R, αS], [2R, αR]), [^{14}C-chlorophenyl CPIA; 4-[^{14}C]-cholesterol; 4-[^{14}C]-cholesteryl-oleate; [^{14}C]-oleic acid; [^{14}C]-lecithin	Miyamoto, J., *et al.* (1986) RAR B.6.1.8
The substrate specificity of microsomal carboxyesterases which form CPIA-cholesterol ester from fenvalerate was investigated by incubating mouse kidney microsomes with ^{14}C-cholesterol and fenvalerate isomers, fenvalerate analogues, other pyrethroids, methoprene and cyclopropate analogues. The same compounds were administered in diet to male mice for 1 month after which the histopathology of the relevant tissues was determined.	Of the fenvalerate isomers, only the [2R,αS]-isomer produced a cholesterol ester. Some fenvalerate analogues produced cholesterol ester conjugates; the other pyrethroids and methoprene did not. Some cyclopropane analogues yielded the corresponding cholesterol ester. Cholesterol ester formation *in vitro* from the fenvalerate analogues correlated well with granuloma formation observed *in vivo* when the analogues were fed to mice at 3000 ppm for 1 month	Test substances: Four [^{14}C-chlorophenyl] chiral isomers of fenvalerate ([2S, αS]; [2S, αR], [2R, αS], [2R, αR]), plus a range of fenvalerate and cycloprate analogues and other pyrethroids	Kaneko, H., *et al.* (1988) RAR B.6.1.8
The oral absorption of esfenvalerate was reassessed.	The oral absorption was calculated to be 64% of the administered dose.		EFSA (2014), RAR B.6.1.9

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

A number of studies are available which examine the ADME of single and repeat oral doses of esfenvalerate and/or fenvalerate in rats and/or mice. The metabolism of fenvalerate has been examined in dogs, and placental transfer of esfenvalerate has been investigated in pregnant rats. Further investigative studies have been conducted in rats and mice to characterise the formation of 'CPIA cholesterol ester'.

The following summary (which focuses on esfenvalerate) is adapted from that in the RAR, and is relevant primarily to the consideration of neurological effects and carcinogenicity.

Absorption

According to the RAR, the oral absorption of esfenvalerate is 64% (based on non-cannulated rats). Absorption was rapid with maximum concentrations of parent or total radioactivity (Tmax) reached within 1-3 hours of dosing, which declined rapidly with the maximum plasma concentration halving (Cmax/2) in <6 hours. Similar TK patterns were observed in the rat, pregnant rat and dog.

Distribution

Esfenvalerate was distributed widely throughout the body. Tissue residues were generally very low.

Metabolism

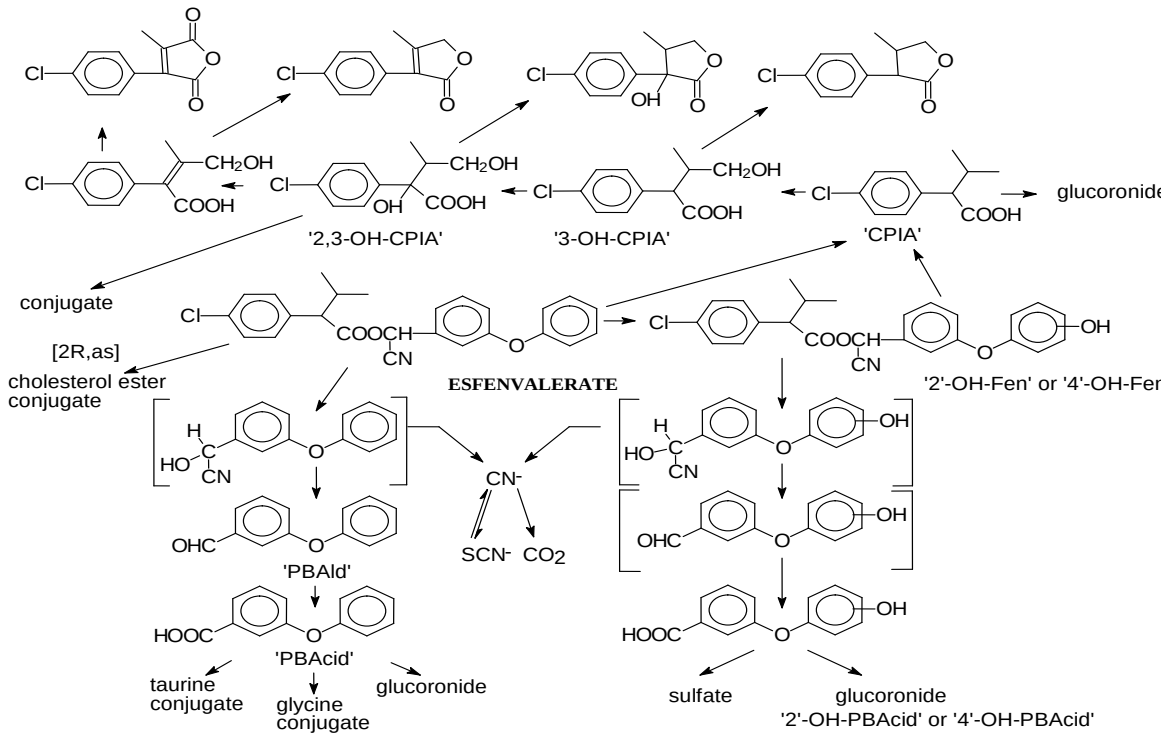
Esfenvalerate was extensively metabolised and more than 20 metabolites were identified. The major radioactive products in the faeces were un-metabolised esfenvalerate and two ester metabolites ('2'-OH'-Fen' and '4'-OH-Fen'). The major urinary metabolites were chlorophenylisovaleric acid ('CPIA'), 3-phenoxybenzoic acid ('PBacid') SCN-, and/or products of further oxidative and conjugation reactions.

The significant metabolic reactions were oxidation at the 2- and 4- positions of the acid, and at the 2'- and 4'-positions of the alcohol moiety, cleavage of the ester linkage and conversion of the cyano group to SCN- and CO_2. There were no major sex differences in the metabolism of esfenvalerate.

Excretion

Excretion of esfenvalerate was very rapid in rats and mice with 78 - 95% of the administered label being excreted within one day after dosing and virtually complete elimination occurred by days 6-7. Tissue residues were generally very low. There was no evidence of accumulation of esfenvalerate in the foetal tissue or amniotic fluid of rats.

Figure 1. Metabolic pathways of esfenvalerate in mammals



10 EVALUATION OF HEALTH HAZARDS

The evaluation of health hazards has been carried out using the studies in the RAR, Esfenvalerate - Volume 3 Annex B.6: Toxicology and metabolism, June 2014. Robust study summaries, taken from the RAR, can be found in Annex I of this CLP report. Esfenvalerate TG = technical grade esfenvalerate. All of the studies were conducted according to guideline and in accordance with GLP, unless otherwise stated.

10.1 Acute toxicity - oral route

Acute toxicity

The acute oral and inhalation toxicity of esfenvalerate are evaluated in light of the introduction of the CLP Regulation and changes to the classification criteria since esfenvalerate was last assessed.

Table 10: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, purity	Value LD_{50}	Reference
OECD 401, no deviations GLP Doses: 0, 5, 10, 20, 40, 55, 75, 100, 130 and 180 mg/kg bw, in corn oil	Rat, Sprague-Dawley, 10 males and 10 females	Esfenvalerate TG (87.2%)	88.5 mg/kg bw (males) 88.5 mg/kg bw (females)	Anonymous (1985d) RAR B.6.2.1
OECD 401, no deviations GLP Doses: 0, 5, 15, 50, 70, 100, 140, 200, 280 and 400 mg/kg bw, in 0.5% methyl cellulose solution	Mouse, ICR, 10 males and 10 females	Esfenvalerate TG (87.2%)	320 mg/kg bw (male) 250 mg/kg bw (female)	Anonymous (1986a) RAR B.6.2.1

Table 11: Summary table of human data on acute oral toxicity

There are no relevant human data available.

Table 12: Summary table of other studies relevant for acute oral toxicity

Type of study	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Acute oral neurotoxicity, rat (Sprague Dawley)	Esfenvalerate TG (purity not reported), 0, 1.75, 1.90, 20, 80 mg/kg in corn oil, 10/sex/group	Groups of 10 males and 10 females given single oral dose at 0, 1.75, 1.90, 20 or 80 mg/kg bw	No mortalities up to 80 mg/kg bw	Anonymous (2000a) RAR B.6.7
Acute oral neurotoxicity, rat (Sprague-Dawley)	Esfenvalerate TG (87.2%), 0, 5, 20, 90 mg/kg in corn oil	Groups of 8 or 16/sex/group given single oral dose at 5, 20 or 90 mg/kg bw	Two males and one female died at 90 mg/kg bw. No mortalities at 20 mg/kg bw	Anonymous (1985e) RAR B.6.7

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

Two guideline studies have been conducted on esfenvalerate technical; one in rats, and one in mice.

In the acute oral study in rats, 10 animals per sex per group were given doses of esfenvalerate in 0, 5, 10, 20, 40, 55, 75, 100, 130 or 180 mg/kg bw esfenvalerate in corn oil by gavage. Dose related mortality was observed

from 55 mg/kg bw with 100% mortality at the highest dose. Neurotoxicity is believed to be the primary cause of lethality. Signs of toxicity were observed from 10 mg/kg bw and included muscular fibrillation, tremors, decrease of spontaneous activity, ataxia, limb paralysis, irregular respiration, dyspnoea, salivation, hyper-excitability and choreothetotic syndrome. These signs gradually developed one hour after treatment, however, they had disappeared in all animals within three days. They were typical of the transient clinical signs associated with pyrethroid toxicity. There were no treatment related gross pathological findings in animals surviving to scheduled termination after the 14-day observation period. Gastric haemorrhaging was noted in animals that died during the study.

The acute oral LD_{50} value for esfenvalerate in both male and female rats was 88.5 mg/kg bw. In the study in mice, 10 animals per sex per 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. 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. 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. Gastric haemorrhaging was noted in animals that died during the study.

The acute oral LD_{50} values for esfenvalerate were 320 and 250 mg/kg bw for male and female mice, respectively. In the RAR, a NOEL of 5 mg/kg bw was identified.

The acute oral neurotoxicity studies summarised in Table 12 (and discussed further in Section 10.11) are also potentially relevant for the classification proposal. In the first of these studies, carried out in rats, 2/16 males and 1/16 females died at the top dose of 90 mg/kg/d (thus, the estimated LD_{50} for this study is >90 mg/kg bw). In the second study, also in rats, no mortalities were observed at doses up to 80 mg/kg bw.

In conclusion, the most sensitive species for assessing acute oral toxicity is the rat. The lowest LD50 value in the rat (88.5 mg/kg bw for both males and females) shall be used as the basis for classification.

10.1.2 Comparison with the CLP criteria

The acute oral LD_{50} in the rat of 88.5 mg/kg bw meets the criterion for Category 3 ($50 < LD_{50} \leq 300$ mg/kg bw).

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Acute Tox. 3; H301: *Toxic if swallowed.*
ATE = 88.5 mg/kg bw

10.2 Acute toxicity - dermal route

Not evaluated in this report.

10.3 Acute toxicity - inhalation route

Table 13: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, purity, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
OECD 403, no deviations GLP	Rat, Sprague-Dawley, 10 males and 10 females	Esfenvalerate TG (87.2%) diluted in corn oil, MMAD 0.94 to 1.07 µm	2.40, 13.8, 205, 395, 550 and 1130 mg/m^3 (0.0024, 0.0138, 0.205, 0.395, 0.550 and 1.130 mg/L), 4 hours, whole body exposure	0.48 and 0.57 mg/L - males and females, respectively	Anonymous (1985f) RAR B.6.2.3

Table 14: Summary table of human data on acute inhalation toxicity

There are no human data on acute inhalation toxicity.

Table 15: Summary table of other studies relevant for acute inhalation toxicity

There are no other studies relevant for acute inhalation toxicity.

10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

A guideline and GLP compliant acute inhalation toxicity study was conducted on esfenvalerate technical.

Groups of 10 male and 10 female rats were exposed whole-body to atmospheric concentrations of 0, 2.40, 13.8, 205, 395, 550 and 1130 mg/m^3 of esfenvalerate diluted in corn oil, for 4 hours. The control animals were exposed to compressed air only and another group exposed to corn oil spray alone. The MMAD of the particles ranged from 0.94 to 1.07 µm.

There was 10, 90 and 100% mortality in male rats exposed to concentrations of 395, 550 and 1130 mg/m^3 of esfenvalerate, respectively. Female rats showed 20, 20 and 100% mortality at the same concentrations of test material, respectively. Deaths occurred within 2 hours after termination of exposure. No treatment-related signs of toxicity were evident in rats exposed to a concentration of 2.40 mg/m^3. At 13.8 mg/m^3 some rats showed signs of irregular respiration, however, this had disappeared within one hour after termination of exposure.

Signs of toxicity at high concentrations (≥205 mg/m^3) included hyperpnoea, dyspnoea, nasal discharge, urinary incontinence, hypersensitivity to sound, muscular fibrillation, abnormal gait, decrease of spontaneous activity, ataxia, lachrimation and salivation. These signs are typical of respiratory distress and non- specific general toxicity, and had 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. Autolysis of the intestinal tract was observed in animals that died during the study.

The acute inhalation 4-hr LC_{50} of esfenvalerate was 0.48 and 0.57 mg/L, in male and female rats respectively. The lowest LC_{50} value will be used as the basis for classification.

10.3.2 Comparison with the CLP criteria

The acute inhalation LC_{50} of 0.48 mg/L in male rats meets the criterion for Category 2 (Inhalation (dust/mist) $0.05< LC_{50} \leq 0.5$ mg/l).

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Acute Tox. 2; H330: *Fatal if inhaled*
ATE = 0.48mg/L

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. Pyrethroid insecticides 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).**

10.4 Skin corrosion/irritation

Not evaluated in this report.

10.5 Serious eye damage/eye irritation

Not evaluated in this report.

10.6 Respiratory sensitisation

Not evaluated in this report.

10.7 Skin sensitisation

Table 16: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Induction and challenge	Results	Reference
OECD 406 (Maximisation test) GLP	Hartley guinea-pig, 20 males / group	Esfenvalerate TG (87.2%)	*Induction*: Intradermal* – 25% Topical – 100% *Challenge*: Topical – 100% (*in corn oil)	Slight to moderate erythema in 15/20 (75%) Guinea pigs after 24 hours and in 17/20 (85%) Guinea pigs after 48 hours after challenge – indicative of a sensitisation response No dermal reactions in vehicle only control group challenged with esfenvalerate **Positive**	Anonymous (1986b) RAR B.6.2.6
OECD 406 (Buehler test) Deviations from guideline: reduced group size	Hartley guinea-pig, 10 males / group	Esfenvalerate TG (87.2%)	*Induction*: Topical x 9 – 100% *Challenge*: Topical – 100%	No dermal reactions in the test group **Negative**	Anonymous (1986c) RAR B.6.2.6
OECD 406 (Buehler test) Deviations from guideline: reduced group size	Duncan-Hartley guinea-pig, 5 per sex / group	Esfenvalerate (purity not reported)	*Induction*: Topical x 3 – 100% *Challenge*: Topical – 100%	No dermal reactions in the test group **Negative**	Anonymous (1986d) RAR B.6.2.6

Table 17: Summary table of human data on skin sensitisation

There are no data on skin sensitisation in humans.

Table 18: Summary table of other studies relevant for skin sensitisation

There are no other studies relevant for skin sensitisation.

10.7.1 Short summary and overall relevance of the provided information on skin sensitisation

A guideline and GLP compliant guinea pig maximisation test was conducted on esfenvalerate technical.

A group of 20 male Hartley guinea pigs were given intradermal injections of 0.05 cm^3 on their shorn scapular sites with (a) Freund's complete adjuvant in water, (b) a 25% solution of esfenvalerate in corn oil and (c) a 50:50 mixture of Freund's adjuvant in water and the esfenvalerate preparation. The control groups were treated similarly.

One week after the injections, the same patch of skin was re-shorn, pre-treated with a sodium lauryl sulphate solution and exposed to undiluted esfenvalerate (0.4 cm^3) applied with lint and held in place for 48 hours with an occlusive patch. The control groups were treated similarly with 0.5% DNCB in corn oil (positive control) or corn oil alone (0.4 cm^3).

The test and control animals were challenged topically two weeks after the induction period with esfenvalerate (approximately 0.2 cm^3) applied to the shaved flank area. The test substance was kept in contact with the skin

by means of an occlusive dressing for a period of 24 hours. The irritation responses were recorded 24 and 48 hours after removal of the occlusive dressings. The vehicle only control group animals received the same topical challenge whilst the positive control group was challenged with DNCB.

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 vehicle only control group animals. There were no treatment-related effects on body weight. DNCB caused moderate to severe skin sensitisation reactions in all animals.

Two other guinea pig tests have been conducted following the Buehler method. Both studies deviated from the test guideline in terms of group size (10 instead of the minimum 20 animals per group required by the guideline), and the first study employed a topical 9-induction procedure compared with a 3-induction procedure in the second study. Whilst both studies were negative for skin sensitisation with esfenvalerate, they are considered to be less robust than the guideline compliant maximisation test. Therefore, the positive skin sensitisation result in the maximisation test is considered to take precedence for consideration of classification.

10.7.2 Comparison with the CLP criteria

The results from the maximisation test suggest that classification in Category 1B may be appropriate, based on the observation of a ≥30% response at a >1% intradermal induction dose and the criteria in Table 3.4.4 of Annex I of CLP.

However, according to the ECHA Guidance on the Application of the CLP Criteria (Version 5.0 – July 2017), classification into subcategories is required when data are sufficient. 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 was investigated in the guinea pig maximisation test. Therefore, we cannot exclude the possibility that sensitisation would have occurred at lower induction concentrations (thus fulfilling the criteria for Category 1A). Skin Sensitsation Category 1 is therefore appropriate.

10.7.3 Conclusion on classification and labelling for skin sensitisation

Skin Sens. 1; H317: *May cause an allergic skin reaction*

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.**

10.8 Germ cell mutagenicity

A number of *in vitro* (Table 19) and *in vivo* (Table 20) mutagenicity studies are available for esfenvalerate.

Table 19: Summary table of mutagenicity/genotoxicity tests *in vitro*

Method, guideline, deviations if any	Test substance, purity	Test system and concentrations	Result	Reference
Bacterial reverse mutation, OECD 471[a]	Esfenvalerate TG (87.4%)	*S. typhimurium* strains TA100, TA98, TA1535, TA1537 and TA1538. *E. coli*, strain WP2 uvr A 15 – 5000 µg/plate Positive controls: -S9: methyl-methane sulfonate for TA100; 2-nitrofluorene for TA98 and TA1538; sodium azide for TA1535; 9-aminoacridine for TA1537 and N-ethyl-N'-nitro-N-nitrosoguanidine for WP2 *uvr* A (Esch. coli). +S9: benzeno (a) pyrene for TA100, TA98, TA1537, TA1538 and 2-amino-anthracene for TA1535 and WP2 *uvr* A	Negative ± S9 Positive controls behaved as expected	Kogiso (1985a) RAR B.6.4.1
Chromosome aberration, OECD 473[b]	Esfenvalerate TG (87.4%)	Chinese hamster ovary cells (CHO-K1) 10^{-5} – 5 x 10^{-4} M Positive controls: -S9: Mitomycin C +S9: benzo (a) pyrene	Negative ± S9 Positive controls behaved as expected	Kogiso (1985b) RAR B.6.4.1
Mammalian cell mutation, OECD 476[a]	Esfenvalerate TG (87.4%)	Chinese hamster lung cells (V79) 10^{-5} – 10^{-3} M Positive controls: -S9: N-methyl-N'-nitro-N-nitrosoguanidine +S9: 3-methylchloranthrene	Negative ± S9 Positive controls behaved as expected	Kogiso (1985c) RAR B.6.4.1

Method, guideline, deviations if any	Test substance, purity	Test system and concentrations	Result	Reference
UDS, OECD 482	Esfenvalerate TG (87.4%)	HeLa cells 3 x 10^{-6} – 10^{-3} M -S9: 3-methylchlolanthrene +S9: 4-nitrosoquinoline-1-oxide (4 NQO)	Negative ± S9 Positive controls behaved as expected	Kogiso (1986) RAR B.6.4.1

[a] Deviations: the positive control substances differed from those recommended in the guideline. Kanechlor 400 was used instead of Aroclor 1254 in the preparation of S-9 mix. Study acceptable
[b] Deviations: Kanechlor 400 was used instead of Aroclor 1254 in the preparation of S-9 mix.

Table 20: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Test substance, purity	Species, strain, sex, no/group	Route of administration and dose levels	Result	Reference
Bone marrow micronucleus, OECD 474[a]	Esfenvalerate TG (87.4%) Positive control: Mitomycin C	Mouse, ICR, male, 6/group	Intraperitoneal injection 40, 80, 150 mg/kg bw	Negative Positive control behaved as expected	Anonymous (1985g) RAR B.6.4.2

[a] Deviations: Justification for using a single sex (males) is not provided. This should not be considered to be a significant deficiency as there is no clear evidence of gender differences in esfenvalerate toxicity.

Table 21: Summary table of human data relevant for germ cell mutagenicity

There are no relevant data in humans.

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

The genotoxicity of esfenvalerate has been adequately investigated in standard tests. Esfenvalerate tested negative in *in vitro* assays for gene mutations, clastogenicity and unscheduled DNA synthesis. Esfenvalerate also tested negative in an *in vivo* micronucleus test. It is therefore concluded that esfenvalerate is not genotoxic.

10.8.2 Comparison with the CLP criteria

All of the available studies were negative, therefore the criteria for classification are not met.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Data conclusive, but not sufficient for classification.

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.**

10.9 Carcinogenicity

The carcinogenicity of esfenvalerate has been investigated in a chronic rat study and in a chronic mouse study. These studies are summarised in Table 22. Other studies which may facilitate the assessment of carcinogenicity are summarised in Table 25.

10.9.1 Carcinogenicity studies

Table 22: Summary table of key animal studies on carcinogenicity with esfenvalerate

<table>
<tr><th>Species (strain), study, guideline</th><th>Remarks and findings of major toxicological significance</th></tr>
<tr><td colspan="2">Rats</td></tr>
<tr><td>2 year combined chronic toxicity/ carcinogenicity study (OECD 453)

Rat (HanRcc:Wistar)

Oral (dietary, pelleted)

Esfenvalerate TG, (87.3%),

0, 15, 50, 150, 400 ppm (~0.7, 2.3, 6.9, 18.5 mg/kg/d)

50/sex/dose (main study group), plus 20/sex/dose (satellite group – killed at 52 weeks)

GLP

Anonymous (2011a)

RAR B.6.5.1</td><td>General toxicity

No adverse effects on survival or clinical signs

Reduced body weights in males at 400 ppm (↓9.7%)

Reduced hindlimb grip strength at 400 ppm (↓10.1% in males, ↓26.9% in females)

Non-neoplastic findings
No findings of major toxicological significance

Neoplastic findings
Tumour types showing statistically significant differences, incidence (no. of affected animals/no. examined) in main study animals. 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.
<table>
<tr><th rowspan="2">Tissue & Tumour Type</th><th></th><th colspan="5">Dietary concentration of esfenvalerate (ppm)</th><th rowspan="2">Historical Control</th></tr>
<tr><th></th><th>0</th><th>15</th><th>50</th><th>150</th><th>400</th></tr>
<tr><td>Testes: Leydig cell tumours (benign)</td><td>Males</td><td>2/50 [4%]</td><td>1/27 [4%]</td><td>0/17 [0%]</td><td>4*/15 [27%]</td><td>4/50 [8%]</td><td>17/628 [2.7%, range 0-4%]</td></tr>
<tr><td rowspan="2">Pituitary gland: adenoma pars anterior</td><td>Males</td><td>8/50 [16%]</td><td>17**/27 [63%]</td><td>10**/18 [56%]</td><td>10**/16 [62%]</td><td>13/50 [26%]</td><td>210/626 [33.5%, range 28.0-38.9%]</td></tr>
<tr><td>Females</td><td>20/50 [40%]</td><td>23*/35 [66%]</td><td>25**/31 [81%]</td><td>25**/34 [73%]</td><td>21/50 [42%]</td><td>349/624 [55.9%, range 42.0-71.3%]</td></tr>
<tr><td rowspan="2">Parathyroid glands: adenoma</td><td>Males</td><td>0/38 [0%]</td><td>0/17 [0%]</td><td>1/12 [8%]</td><td>2*/11 [18%]</td><td>1/42 [2%]</td><td>8/532 [1.5%, range 0 – 5.1%]</td></tr>
<tr><td>Females</td><td>0 [0%]</td><td>0 [0%]</td><td>0 [0%]</td><td>0 [0%]</td><td>0 [0%]</td><td>0 [0%]</td></tr>
<tr><td rowspan="2">Thymus; thymoma lymphatic type, benign</td><td>Males</td><td>0/47 [0%]</td><td>1/22 [4%]</td><td>2*/14 [14%]</td><td>1/15 [7%]</td><td>1/45 [2%]</td><td>9/600 [1.5%, range 0 – 4.4%]</td></tr>
<tr><td>Females</td><td>0/48 [0%]</td><td>6**/25 [24%]</td><td>5**/23 [22%]</td><td>5**/17 [29%]</td><td>3/49 [6%]</td><td>22/615 [3.6%, range 0-16%]</td></tr>
<tr><td></td><td>Males</td><td>2/50</td><td>3/24</td><td>1/12</td><td>1/11</td><td>2/50</td><td>9/480</td></tr>
</table></td></tr>
</table>

Species (strain), study, guideline	Remarks and findings of major toxicological significance							
	Haemolymphoreticular system: malignant lymphoma		[4%]	[12%]	[8%]	[9%]	[4%]	[1.9%, range 0 – 3.7%]
		Females	0/50 [0%]	3*/17 [18%]	0/19 [0%]	0/14 [0%]	0/50 [0%]	4/480 [0.8%, range 0-2.0%]
	Mammary gland: fibroadenoma	Males	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0/479 [0%, range 0-0%]
		Females	10/50 [20%]	14**/27 [52%]	19**/29 [66%]	13**/23 [57%]	13/49 [27%]	180/626 [28.8%, range 22-36%]
	Mammary gland: adenocarcinoma	Males	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	1/479 [0.2%, range 0-2%]
		Females	5/50 [10%]	3/27 [11%]	6/29 [21%]	7*/23 [30%]	5/49 [10%]	37/626 [5.9%, range 2.0-12.0%]
	*significantly different from control, $p<0.05$ **significantly different to control, $p<0.01$ Historical control data is from 6-8 chronic (104 weeks) toxicity studies with Wistar rats conducted at Harlan Laboratories, completed between July 2005 and February 2009.							

<table>
<tr><th>Species (strain), study, guideline</th><th>Remarks and findings of major toxicological significance</th></tr>
<tr><td>Histopathological examination of testes in all remaining animals of the 15, 50 and 150 ppm groups in the study detailed above.

Anonymous (2015)</td><td>Incidence of benign Leydig cell tumours and Leydig cell hyperplasia:
<table>
<tr><th>Dose (ppm)</th><th>0</th><th>15</th><th>50</th><th>150</th><th>400</th></tr>
<tr><td>Leydig cell tumours (original histopathology data)</td><td>2/50 [4%]</td><td>1/27 [4%]</td><td>0/17 [0%]</td><td>4*/15 [27%]</td><td>4/50 [8%]</td></tr>
<tr><td>Leydig cell tumours - additional histopathology</td><td>2/50 [4%]</td><td>1/50 [2%]</td><td>0/50 [0%]</td><td>4/50 [8%]</td><td>4/50 [8%]</td></tr>
<tr><td>Leydig cell hyperplasia</td><td>2/50 [4%]</td><td>1/50 [2%]</td><td>0/50 [0%]</td><td>1/50 [2%]</td><td>0/50 [0%]</td></tr>
</table>
* significantly different from control, p<0.05

No malignant Leydig cell tumours were observed in any control or treated animals. There were no toxicologically significant changes in the incidence of Leydig cell hyperplasia</td></tr>
<tr><td colspan="2">Mice</td></tr>
<tr><td>Mouse (Crl:CD), 18-month oral dietary (powdered), OECD 451

Anonymous (1997)

RAR B.6.5.2

Esfenvalerate TG, (84.8%)

0, 35, 150 ppm (4.29, 18.3 mg/kg/d) for 18 months, and 350 ppm for 2 months

80/sex/group</td><td>General toxicity

350 ppm:

All animals in this dose group were sacrificed on days 57 and 58 following excessive self-trauma (induced by effect of substance on dermal sensory nerves)

150 ppm:
M&F: decreased survival attributed to large number of mice sacrificed due to self-mutilation (considered secondary to sensory nerve stimulation due to dermal contact with diet containing test substance)
M&F: decreased body weight / gain

Non-neoplastic findings
No toxicologically significant findings

Neoplastic findings
No treatment related tumours

NOAEL identified in the RAR = 35 ppm (equivalent to a daily intake of 4.3 mg/kg bw/d in males and 5.7 mg/kg bw/d in females)</td></tr>
</table>

Notes: (i) deviations from guidelines are noted in the study summaries presented in Annex 1

Guideline 2 year combined chronic toxicity/ oncogenicity study in rats

70 Wistar rats/sex/dose were fed a diet containing esfenvalerate at a concentration of 0, 15, 50, 150 or 400 ppm. The dose levels were selected based on the results of 4 week and 13 week 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); 20 rats/sex/dose formed a 'satellite' group (sacrificed after 52 weeks). A FOB and locomotor activity (observed over a 60 min time period) measurements were conducted at week 48 for all satellite animals.

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).

A significant reduction in hindlimb grip strength was noted in both sexes at the top dose. Forelimb grip strength was not affected by treatment. In the absence of any related histopathological findings in skeletal muscle, sciatic nerve and lumbar spinal cord, the hindlimb grip strength findings are of uncertain toxicological significance.

In the locomotor activity assessment, there was a dose-related decrease in total activity among treated males over the 60 minute observation period. However, the effect was not statistically significant.

The number of males at 400 ppm (main study) with spinal cord radiculoneuropathy was significantly increased. However, this is a normal background lesion with a wide range of natural variation (historical control incidence range 0 – 96%) and the incidences at 400 ppm 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 considred 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.

The overall incidence of animals with benign and/or malignant tumours was similar in all groups. 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 22.

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 it can be concluded that there is no evidence of carcinogenic activity in the pituitary, the haemolymphoreticular system, thymus or mammary gland.

The percentage of animals (male) with benign Leydig cell tumours was greater than controls in the 150 and 400 ppm treatment groups. The effect was statistically significant at 150 ppm only.

Additional histopathology report

The EFSA peer review of esfenvalerate suggested that a classification of Carcinogenicity Category 2 may be appropriate, based on the incidence of Leydig cell tumours in the testes of male rats in the carcinogenicity study discussed above (EFSA, 2014). 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. The results of this additional investigation can be found in Table 22. The Applicant has also provided an analysis of the data, which can be found in Appendix 1. This additional histopathological examination was not available for EFSA peer review before the renewal of the approval decision.

The evaluation of additional testes sections 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.

Historical control data have been provided by the Applicant, and are detailed in Tables 23 and 24.

Table 23: Historical background values of testicular Leydig cell tumours in male Wistar rats from 2-year feeding studies at Harlan Laboratories (provided by the Applicant) completed during the 5 years prior to Anonymous, 2011 being conducted.

Study ID #	**41**	**43**	**44**	**45**	**48**	**50**	**51**	**53**	**Current study**
Year completed	2005	2006	2006	2006	2005	2006	2008	2009	2011
Pathologist	WEK	WEK	WEK	KHE	HJC	WEK	WEK	WEK	
Number of rats examined	50	112	100	99	107	50	50	100	50
Leydig cell tumour (benign)	0	4	1	2	4	0	1	4	2
%	0.0	3.6	1.0	2.0	**3.7**	**0.0**	**2.0**	**4.0**	**4.0**
Leydig cell tumour (malignant)	0	0	0	0	0	0	0	0	0
%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Leydig cell hyperplasia	0	2	1	1	0	0	0	1	2
%	0.0	1.8	1.0	1.0	0.0	0.0	0.0	1.0	4.0

Table 24: Additional historical background values of testicular Leydig cell tumours in male Wistar rats from 2-year feeding studies at Harlan Laboratories (provided by the Applicant) from studies completed more than 5 years prior to Anonymous, 2011 being conducted.

Study ID #	**2**	**3**	**6**	**8**	**14**	**24**	**32**	**33**	**34**	**35**	**36**	**37**	**39**	**40**	**42**
Year completed	1983	1985	1985	1986	1987	1989	1994	1995	1997	1996	1996	1999	2003	2004	2004
Pathologist	JMA	RUD	HHW	JMA	BSC	JMA	JMA	HHW	JMA	HJC	HHW	JMA	WEK	JMA	WEK
Number of rats examined	99	100	50	100	60	70	50	100	99	50	60	64	70	50	50
Leydig cell tumour (benign)	0	5	1	2	1	7	5	3	9	2	0	1	0	0	0
%	0.0	5.0	2.0	2.0	1.7	10.0	10.0	3.0	9.1	4.0	0.0	1.6	0.0	0.0	0.0
Leydig cell tumour (malignant)	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4	0.0	0.0

Leydig cell hyperplasia	1	1	0	3	5	3	11	0	4	0	0	3	0	3	1
%	1.0	1.0	0.0	3.0	8.3	4.3	22.0	0.0	4.0	0.0	0.0	4.7	0.0	6.0	2.0

The incidences of benign Leydig cell tumours at 150 and 400 ppm (8%) were not statistically significant; however, they were above the level of the concurrent control (4%). They were also outside the range of the historical control data collected during the 5 years prior to the study being conducted (0 – 4%, see Table 23). Therefore, consideration should be given as to whether the increase in Leydig cell tumours seen in rats dosed with esfenvalerate was treatment-related. A comprehensive assessment of the tumours has been performed by the Applicant, and is provided in Appendix 1.

Consideration of whether the benign Leydig cell tumours in the 2 year rat study are treatment-related
In this study, the incidence of benign Leydig cell tumours at the top two doses was greater than controls (4, 2, 0, 8 and 8% at 0, 15. 50, 150 and 400 ppm), however 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. Although older historical control data should be treated with caution, it is worth noting that control incidences of 9.1, 10.0 and 10.0% were reported in the same laboratory 14, 17 and 22 years prior (see Table 24). In addition, there was no temporal trend in the backround incidence of Leydig cell tumours, which supports the comparison with the control incidences between 1983 and 2004.

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 (see Section 10.12) and reproductive toxicity studies (see study summaries in Annex 1) on esfenvalerate, there were no findings which were indicative of an adverse effect on the testes or the endocrine system.

Overall, the slight increase in benign Leydig cell tumours seen at the top two doses in the rat carcinogenicity study is not considered to be treatment-related. The result is not statistically significant or biologically significant, and is therefore not relevant for classification.

Guideline 18 month dietary study in mice

Crl: CD mice (80/sex/dose) were fed diets containing 0, 35, 150 or 350 ppm of esfenvalerate for 18 months. Mice in the 350 ppm group developed excessive morbidity and mortality due to self trauma induced by the pharmacological effects of the test substance on dermal sensory nerves and were sacrificed by design on test days 57 and 58.

Survival was significantly decreased in males (46%, compared to 70% in controls) and females (41%, compared to 71% in controls) in the 150 ppm group, 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 present in these animals 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 pharmacological

effects of 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.

Table 25: Summary table of human data on carcinogenicity

There are no relevant data in humans.

10.9.2 Supporting information for the assessment of carcinogenicity

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 (EDSP). These studies are briefly summarised in Table 26 (further information can be found in Annex I) to support the assessment of carcinogenicity.

Table 26: Summary Mechanistic studies conducted as part of the US EPA's 'Endocrine Disruptor Screening Program'.

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) RAR B.6.5.3	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) RAR B.6.8.3	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) RAR B.6.8.3	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

Type of study, guideline	**Test substance, purity**	**Relevant information about the study (as applicable)**	**Observations**
In vitro estrogen receptor transcriptional activation, OECD 455 Anonymous (2012b) RAR B.6.8.3	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-9903 cell line. Concentrations: $10^{-10.6}$ to $10^{-3.6}$M Positive control: 17β-estradiol in DMSO	No agonist activity The positive control behaved as expected.
In vitro H295R steroidogenesis assay, OECD 456 Anonymous (2012c) RAR B.6.8.3	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) RAR B.6.8.3	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-^{3}H]-Androst-4-ene-3,17-dione, [^{3}H]-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

All of the mechanistic studies were negative, and do not provide any evidence of a carcinogenic potential of esfenvalerate.

10.9.3 Short summary and overall relevance of the provided information on carcinogenicity

Data on esfenvalerate are available from guideline carcinogenicity studies conducted in rats and mice via the oral route.

In a 2 year study in HanRcc: Wistar rats, there was a slight increase in the incidence of benign Leydig cell tumours at the top two doses (6.9 and 18.5 mg/kg bw/d) compared to the concurrent control. The increase was not statistically significant or biologically significant. In an 18 month study in mice, no treatment-related tumours were reported at doses of up to 18.3 mg/kg bw/d (although it is noted that all mice in the top dose group were sacrificed early in this study following excessive trauma and self-mutilation).

Esfenvalerate was negative in standard *in vitro* and *in vivo* tests for genotoxicity. It also tested negative in a range of mechanistic studies conducted to investigate the endocrine disrupting potential of esfenvalerate. Overall, the available data do not provide any evidence that esfenvalerate is carcinogenic.

10.9.4 Comparison with the CLP criteria

There is no evidence that esfenvalerate is carcinogenic. No classification is proposed.

10.9.5 Conclusion on classification and labelling for carcinogenicity

Not classified – conclusive but not sufficient for classification.

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 activation, OECD 455 Anonymous (2012b)	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-9903 cell line. Concentrations: 10-10.6 to 10-3.6M Positive control: 17β-estradiol in DMSO	No agonist activity The positive control behaved as expected.
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	No significant inhibition of aromatase activity up to the limit of solubility The positive controls behaved as expected

		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%	

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**.

10.10 Reproductive toxicity

Not considered in this report.

10.11 Specific target organ toxicity-single exposure

The most relevant studies for consideration of STOT SE are the acute neurotoxicity studies conducted on esfenvalerate (Table 27). Other studies of potential relevance are summarised in Table 29. The study summaries are presented in Annex 1.

Table 27: Summary table of animal studies on STOT SE

Species (strain), study, guideline	**Test substance (purity), dose levels, no./sex/group**	**Remarks and findings of toxicological significance**	**Reference**
Rat (Sprague-Dawley), acute oral neurotoxicity, OECD 424	Esfenvalerate TG (purity not reported), 0, 1.75, 1.90, 20,80 mg/kg, 10/sex/group	The animals received a single gavage dose of esfenvalerate dissolved in corn oil and were observed for up to 16 days. The observations included clinical examinations, functional observational battery (FOB) and motor activity measurements. Neurohistopathology was conducted at termination. There was no mortality up to 80 mg/kg. No treatment-related effects observed at 1.75 mg/kg **1.9 mg/kg** Tremors in one female **20 mg/kg** Stereotypical grooming and tremors in occasional animals **80 mg/kg** Number of transient changes, namely changes in clinical condition (including 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 footsplay, reduced body weight gain and reduced food consumption. No microscopic neurological lesions observed at any dose level All clinical signs of reaction to treatment had resolved by 4 days after dosing. NOAELs (taken from the RAR) were 1.9 mg/kg for males and 1.75 mg/kg for females	Anonymous (2000a) RAR B.6.7
Rat (Sprague-Dawley), acute oral neurotoxicity, OECD 424[1]	Esfenvalerate TG (87.2%), 0, 5, 20, 90 mg/kg or fenvalerate (95.5%), 20, 80, 360 mg/kg 8 or 16/sex/group	The animals received a single gavage dose of esfenvalerate or fenvalerate dissolved in corn oil and were observed for 2 weeks. The observations included clinical examinations and functional testing using inclined plane (slip angle test). Neurohistopathology was conducted at termination. Two males and one female receiving 90 mg/kg esfenvalerate and one male and four females receiving 360 mg/kg fenvalerate, were found dead within 24 hours of dosing. Clinical signs of toxicity; from 2 hours after dosing such as muscular fibrillation, hunched posture and ataxia were observed in the intermediate and high dose groups with both compounds. Tremor and limb paralysis were also observed in some animals from the high dose groups. No treatment related clinical signs of toxicity were observed at doses of up to20 mg/kg fenvalerate. All clinical signs of toxicity had resolved within 2 days of dosing.	Anonymous (1985e) RAR B.6.7.1

Species (strain), study, guideline	Test substance (purity), dose levels, no./sex/group	Remarks and findings of toxicological significance	Reference
		Slight to minimal axonal degeneration and/or demyelination with Schwann cell proliferation in peripheral nerves were noted for both compounds at the highest doses. No pathological lesions were observed at non-lethal doses where neurological clinical signs were present. NOAELs (taken from the RAR) were 5 mg/kg and 20 mg/kg for esfenvalerate and fenvalerate, respectively.	

[1] Detailed clinical observations and functional testing appeared not to be as comprehensive as specified in the test guideline

Table 28: Summary table of human data on STOT SE

There are no relevant data in humans.

Table 29: Summary table of other studies relevant for STOT SE

Type of study	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Acute oral rat, OECD 401 Sprague Dawley rats, 10/sex/dose	Esfenvalerate TG (87.2%)	Dose levels: 0, 5, 10, 20, 40, 55, 75, 100, 130 and 180 mg/kg bw/d (in corn oil)	**5mg/kg** No treatment-related effects **10 mg/kg** Transient muscular fibrillation and decrease of spontaneous activity **40 mg/kg** Transient muscular fibrillation, occasional signs of tremor, limb paralysis and ataxia **55 mg/kg and above** Mortality (10, 40, 50, 90 and 100% in both sexes dosed with 55, 75, 100, 130 and 180 mg/kg) Transient signs of toxicity included muscular fibrillation, tremors, decrease of spontaneous activity, ataxia, limb paralysis, irregular respiration, dyspnoea, salivation, hyper-excitability and choreoathetotic syndrome .These signs gradually developed one hour after dosing, however, they had disappeared in all animals within 3 days and generally within 2 days. NOAEL (taken from the RAR) was 5 mg/kg	Anonymous (1985d) RAR B.6.2.1
Acute oral mouse,	Esfenvalerate	Dose levels: 0, 5, 15, 50, 70, 100, 140, 200, 280 and 400	**5 mg/kg**	Anonymous

Type of study	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
OECD 401 ICR mice, 10/sex/dose	TG (87.2%)	mg/kg in methyl cellulose solution	No treatment-related effects **15 mg/kg** Transiet muscular fibrillation and decrease of spontaneous activity **70 mg/kg & 100 mg/kg** Transient muscular fibrillation, occasional signs of tremor, limb paralysis and ataxia **140 mg/kg and above** Mortality: 10, 30, 60 and 90% mortality with female mice dosed 140, 200, 280 and 400 mg/kg respectively; 20, 30 and 100% mortality in male mice dosed 200, 280 and 400 mg/kg respectively. 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 had disappeared in all surviving animals within 2 days. NOAEL (taken from the RAR) was 5 mg/kg	(1986a) RAR B.6.2.1
Acute dermal rat, OECD 402 Sprague Dawley rats, 10/sex/dose	Esfenvalerate TG (87.2%)	Dose levels: 0, 500, 1000, 2000, 3200 and 5000 mg/kg in corn oil	No mortality. **500 mg/kg** No treatment-realted effects **1000 mg/kg** Transient muscular fibrillation **2000 mg/kg and above** Transiet muscular fibrillation, decrease of spontaneous activity, ataxia, irregular respiration and urinary incontinence. These signs of toxicity developed 2 - 4 hours after application but had disappeared within 8 days. NOAEL (taken from the RAR) was 500 mg/kg	Anonymous (1985m) RAR B.6.2.2
Acute dermal rabbit, OECD 402 New Zealand White rabbits,	Esfenvalerate TG (87.2%)	Dose level: 2000 mg/kg, undiluted. Study not acceptable at the AIR2 Renewal Review because of 20% mortality in the vehicle (water) control group	No treatment related mortality. 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.	Anonymous (1985j) RAR B.6.2.2

Type of study	Test substance	Relevant information about the study (as applicable)	Observations	Reference
5/sex/group				
Acute inhalation rat, OECD 403 Sprague Dawley rats, 10/sex/dose	Esfenvalerate TG (87.2%)	Exposure concentrations: 0, 2.40, 13.8, 205, 395, 550 and 1130 mg/m^3 in corn oil	**2.40 mg/m^3** None **13.8 mg/m^3** Some rats showed irregular respiration, but this disappeared within 1 hour after termination of exposure **205 mg/m^3 and above** Mortality: 10, 90 and 100% in males and 20, 20 and 100% in females exposed to 395, 550 and 1130 mg/m^3. 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 had 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. NOAEC (taken from the RAR) was 2.40 mg/m^3 (0.0024 mg/L)	Anonymous (1985f) RAR B.6.2.3

10.11.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure

Two acute oral neurotoxicity studies are available in rats. In both studies, transient signs of toxicity indicative of neurological effects in FOB and motor activity measurements were observed at doses ≥20 mg/kg. There were no irreversible adverse effects at sub lethal doses. In Anonymous (1985e), neuropathological lesions were observed at the highest doses of esfenvalerate which resulted in mortality. This study was not fully compliant with the guideline and the GLP status was not reported. In the later study (Anonymous, 2000a), which was compliant with the guideline, microscopic examination of the nervous system did not reveal any treatment-related changes.

In acute oral toxicity studies in rats and mice, similar transient signs of toxicity were seen in both species (e.g., muscular fibrillation, tremors, decrease of spontaneous activity, ataxia, limb paralysis, irregular respiration, salivation), leading to death at higher doses. Similar effects were observed in an acute inhalation study in rats. Similar effects were also observed in acute dermal studies in rats and rabbits, although no treatment-related mortalities were observed in these studies (up to doses of 2000 mg/kg in the rabbit and 5000 mg/kg in the rat). There were no gross pathological findings indicative of target organ toxicity in any of these studies.

Esfenvalerate is a synthetic pyrethroid insecticide. The significant acute toxic effects of esfenvalerate consisted primarily of neurological signs consistent with the known mode of action of pyrethroids (see Soderlund et al, 2002, for a comprehensive review). Pyrethroid insecticides act on the sodium channel in the nerve membranes of the invertebrate nervous system and are termed sodium channel modulators. They cause pronounced

repetitive activity and 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. 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.

10.11.2 Comparison with the CLP criteria

Specific target organ toxicity (single exposure) is defined as specific, non-lethal target organ toxicity arising from a single exposure to a substance or mixture.

STOT SE 3

STOT SE Cat 3 only includes narcotic effects and respiratory tract irritation. Section 3.8.2.2.2 of Annex I of the CLP Regulation states that *"narcotic effects observed in animal studies may include lethargy, lack of coordination, loss of righting reflex, and ataxia. If these effects are not transient in nature, then they shall be considered to support classification for Category 1 or 2 specific target organ toxicity single exposure".* Transient neurological signs observed with esfenvalerate include ataxia, un-coordination, abnormal gait, slow righting reflex, reduced motor activity, reduced forelimb grip strength and hind limb footsplay, tremor, limb paralysis and muscular fibrillation. Some of these signs are indicative of narcosis, however, this is not the case for tremor, limb paralysis and muscular fibrillation. Overall, the profile of effects seen with esfenvalerate is considered not to be indicative of narcosis, and classification in STOT SE Cat 3 is therefore not appropriate.

With regards respiratory tract irritation, respiratory irritant effects (characterised by localised redness, oedema, pruritis and/or pain) that impair function with symptoms such as cough, pain, choking and breathing difficulties are included. The evaluation is based primarily on human data, however no human data are available for esfenvalerate. Section 3.8.2.2.1 of Annex I of CLP states "*there are currently no validated animal tests that deal specifically with RTI, however, useful information may be obtained from the single and repeated inhalation toxicity tests. For example, animal studies may provide useful information in terms of clinical signs of toxicity (dyspnoea, rhinitis etc) and histopathology (e.g. hyperemia, edema, minimal inflammation, thickened mucous layer) which are reversible and may be reflective of the characteristic clinical symptoms described above. Such animal studies can be used as part of a weight of evidence evaluation.*" In an acute inhalation study in rats, some animals showed signs of irregular respiration; however this had disappeared within one hour after termination of exposure. Histopathological examination of the nasal cavity, trachea and lungs did not reveal any treatment-related findings. Therefore, classification in STOT SE Cat 3 for respiratory tract irritation is not appropriate based on the available data.

STOT SE Cat 1 and Cat 2

According to Table 3.8.1 in Annex I of CLP, STOT SE 1 is reserved for, "*substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following single exposure*". Category 2 is reserved for, "*substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following single exposure*." In acute oral toxicity and acute oral neurotoxicity studies in rats and mice, significant and severe signs of toxicity (neurological effects, death) were observed at doses relevant for classification for STOT SE (i.e., ≤ 2000 mg/kg bw). The severity of the neurological effects increased in a dose-dependent manner, and neurotoxicity is considered to be the primary cause of lethality. Lethality occurred at doses relevant for classification for acute toxicity, therefore classification for Acute Tox 3 (H301) has been proposed. Care must be taken not to assign STOT SE and acute toxicity for the same toxic effect (leading to a 'double classification'). Therefore, given that esfenvalerate is already classified for acute toxicity by the oral route (based on deaths caused by neurotoxicity), it is not appropriate to classify for STOT SE 1 or 2 based on neurotoxic effects.

In an acute inhalation study in rats, significant and severe signs of toxicity (neurological effects, death) were observed at doses relevant for classification for STOT SE (≤ 5 mg/l/4h). As in the oral studies discussed above, the severity of the neurological effects increased in a dose-dependent manner, and neurotoxicity is considered to be the primary cause of lethality. Lethality occurred at doses relevant for classification for acute toxicity, and classification as Acute Tox 2 (H330) has been proposed. Therefore, classification for STOT SE is not considered appropriate, as it would result in a double classification.

In an acute dermal toxicity study in the rat, transient neurological effects were confined to muscular fibrillation at 1000 and 2000 mg/kg bw, and occasional decrease of spontaneous activity and ataxia at 2000 mg/kg bw. No mortality was observed up to the highest dose level (5000 mg/kg bw). These relatively minor transient neurological signs are 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 in a rabbit acute dermal toxicity studyconsisted of decreased activity, ataxia, body tremors, constricted pupils, muscle tremors and poor hind limb co-ordination in the presence of other significant general toxicity. The reliability of this study is questionable, due to 20% mortality in the vehicle (water) control group. Overall, the results of the acute dermal studies do not support classification in STOT SE 1 or 2.

10.11.3 Conclusion on classification and labelling for STOT SE

Not classified – conclusive but not sufficient for classification.

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 picture, 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.

10.12 Specific target organ toxicity-repeated exposure

The repeated dose toxicity of esfenvalerate has been investigated in standard studies in rats (one 28 day dose range finding study, two 90 day oral studies, two 90 day oral neurotoxicity studies and a 21 day dermal study), mice (one 90 day oral study) and dogs (a 1 year oral study). The results of these studies are summarised in Table 30.

Table 30: Summary table of animal studies on STOT RE

Species (strain), study, guideline	Test substance (purity), dose levels, no./sex/group	Remarks and findings of toxicological significance	Reference
Oral Route			
Rats			
28 day dietary, OECD 407 Non-GLP Rat (Wistar: HanRcc: WIST (SPF)) Guidance values: Cat 1: C ≤ 30 Cat 2: 30 < C ≤ 300	Esfenvalerate TG (87.3%), 0, 300, 500, 700 and 1000 ppm (in pelleted diet) Equivalent to 0, 22.0, 35.4,46.0 and 44# mg/kg bw/d in males and 0, 23.1, 39.8, 54.0 and 46.5# mg/kg bw/d in females, 10/sex/dose	300 ppm - 22.0 / 23.1 mg/kg bw/d No toxicologically significant findings 500 ppm - 35.4 / 39.8 mg/kg bw/d Clinical signs: abnormal/swaying gait and muscle twitching Reduced food consumption (↓14.6% in males) and body weight (↓11.6% in males) 700 ppm - 46.0 / 54.0 mg/kg bw/d Mortality: 1 male died spontaneously on day 28	Anonymous (2008) (Study not reported in the RAR but provided by industry for the purposes of this report)

Species (strain), study, guideline	Test substance (purity), dose levels, no./sex/group	Remarks and findings of toxicological significance	Reference
	# values recorded after 1 week of treatment	Clinical signs: stiff gait (1 female), ataxia (5 females), abnormal/swaying gait and muscle twitching Reduced food consumption (↓26.8% in males, ↓9.6% in females) and body weight (↓19.7% in males, ↓14.0% in females) 1000 ppm – 44.0 / 46.5 mg/kg bw/d Mortality: 7 males died between day 7 and 12. 2 males had to be killed in extremis on day 11 and the remaining male on day 12. 2 females died spontaneously on day 7 and 2 females died spontaneously on day 8. The remaining 6 females were killed in extremis on day 8. Clinical signs: ataxia (all individuals), aggressive behaviour (1 female) and vocalisation when touched (1 female), prostration (1 female), abnormal/swaying gait and muscle twitching Reduced food consumption (↓71.8% in males, ↓56.2% in females) and body weight (↓23.6%# in males, ↓21.3%# in females) Macroscopic findings: dark red discoloured lungs (4 males, 2 females); dark red discoloured lungs and thymus (1 male) # values recorded after 1 week of treatment (deaths of animals prevented any further measurement)	
90-day dietary, OECD 408 Rat (Sprague-Dawley) Guidance values: Cat 1: C ≤ 10 Cat 2: 10 < C ≤ 100	Esfenvalerate TG (purity not reported), 0, 50, 150, 300, 500 ppm (0, 2.5, 7.5, 15.0, 25.0 mg/kg/d) 30/sex/group Groups treated for 7 or 13 weeks.	2.5 mg/kg bw/d No toxicologically significant findings7.5 mg/kg bw/d One animal exhibited jerky leg movements 15.0 mg/kg bw/d Jerky leg movements, unsteady gait Slight to moderate hypertrophy of the parenchymal cells in the parotid salivary gland and with lower incidence in the pituitary glands 25.0 mg/kg bw/d Some females died or became moribund during the study. See text below and Table 31 for further information.	Anonymous (1984) RAR B.6.3.1

Species (strain), study, guideline	Test substance (purity), dose levels, no./sex/group	Remarks and findings of toxicological significance	Reference
		Jerky leg movements, unsteady gait, body tremors, hypersensitive to sounds, convulsions The signs were usually observed from within the first few weeks of dosing to termination in the high dose group. Slight to moderate hypertrophy of the parenchymal cells in the parotid salivary gland and with lower incidence in the pituitary glands. NOAEL (taken from RAR) = 50 ppm (~2.5 mg/kg/d).	
90-day dietary, OECD 408 Rat (Sprague-Dawley) Guidance values: Cat 1: C ≤ 10 Cat 2: 10 < C ≤ 100	Esfenvalerate TG (purity not reported), 0, 75, 100, 125, 300 ppm (0, 3.75, 5.0, 6.25, 15 mg/kg/d) 25/sex/group Groups treated for 7 or 13 weeks. No microscopic evaluation as no treatment related findings were noted below 300 ppm in the previous study (reported above).	6.25 mg/kg bw and below No toxicologically significant findings15 mg/kg bw/d Neurological signs beginning 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. Higher absolute and relative kidney weights at 300 ppm. Increased relative liver weights at 125 and 300 ppm were considered to be adaptive. NOAEL (taken from the RAR) = 125 ppm (~6.25 mg/kg/d)	Anonymous (1987) RAR B.6.3.1
90-day dietary neurotoxicity study, OECD 424 Rat (Sprague-Dawley) Guidance values: Cat 1: C ≤ 10 Cat 2: 10 < C ≤ 100	Esfenvalerate TG (purity not reported), 0, 50, 100, 300 ppm (0, 3.2, 6.4, 20.1 mg/kg/d) 12/sex/group 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.	3.2 mg/kg bw/d No toxicologically significant findings6.4 mg/kg bw/d Reduced body weight gain in males (↓10.4%) 20.1 mg/kg bw/d 2 unscheduled deaths (males: killed early due to serious skin sores, day 52 and day 88) Reduced bodyweight (males: ↓12.5%, females ↓10%) Reduced body weight gain (males: ↓19.3%, females: ↓20.7%) Reduced food consumption in males (↓7%) Abnormal gait (males and females) Reduction in forelimb grip strength (in males at week 4 and 8; in females at week 4)	Anonymous (2000c) RAR B.6.7.1

Species (strain), study, guideline	Test substance (purity), dose levels, no./sex/group	Remarks and findings of toxicological significance	Reference
		Reduction in hindlimb grip strength (in males at 4 weeks; in females 4 and 13 weeks) Reduced footsplay (in males only, at week s 4 and 8) There were no microscopic neurological lesions observed at any dose level NOAEL (taken from the RAR) = 50 ppm (3.2 mg/kg/d) for males and 100 ppm (7.3 mg/kg/d) for females.	
Rat (Sprague-Dawley) 90-day dietary neurotoxicity study, OECD 424 Guidance values: Cat 1: C ≤ 10 Cat 2: 10 < C ≤ 100	Esfenvalerate TG (97.3% total isomers, 86.0% as esfenvalerate), 0, 40, 120, 360 ppm (0, 3.0, 8.9, 28.8 mg/kg/d) 12/sex/group The observations included clinical examinations, functional observational battery (FOB) and motor activity measurements (pre-dose, weeks 2, 5, 9 and 13). Neurohistopathology was conducted at termination.	8.9 mg/kg bw/d Significant decrease in total activity counts (females only) at week 2 28.8 mg/kg bw/d Skin ulcerations (males) Reduced body weight (males and females) Forelimb grip strength significantly reduced (males and females), and reduced ease of removal from home cage (females only) at week 2 Significant decrease in total activity counts (females only) at week 2 There were no microscopic neurological lesions at any dose. NOAEL 40 ppm (3.0 mg/kg/d)	Anonymous (1999c) RAR B.6.7.2
Mouse			
Mouse (B6C3F1) 90-day dietary, OECD 408 Guidance values: Cat 1: C ≤ 10 Cat 2: 10 < C ≤ 100	Esfenvalerate TG (purity not reported), 0, 50, 150, 500 ppm (10.5, 30.5, 106 mg/kg/d) 12/sex/group.	Treatment related clinical signs at 500 ppm (~106 mg/kg/d) 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 at 500 ppm. Histopathological findings at 500 ppm included inflammatory changes in skin; reactive changes in lymphatic tissues, slight ulcerative changes in stomach and decrease of fat deposition	Anonymous (1985h) RAR B.6.3.2

Species (strain), study, guideline	Test substance (purity), dose levels, no./sex/group	Remarks and findings of toxicological significance	Reference
		in liver and kidneys (correlated with lower plasma lipids). NOAEL (taken from the RAR) = 150 ppm (~30.5 mg/kg/d)	
Dog			
Dog (Beagle) one-year oral, OECD 452	Esfenvalerate TG (purity not reported), 0, 25, 50, 100, 200 ppm (0, 0.66, 1.28, 2.58, 5.02 mg/kg/d) 6/sex/group	No toxicologically significant findings. NOEL (taken from the RAR) = 200 ppm (~5 mg/kg/d)	Anonymous (1986e) RAR B.6.3.3
Dermal route			
Rat			
Rat (Sprague-Dawley) 21-day dermal, OECD 410 Guidance values: Cat 1: C ≤ 80 Cat 2: 10 < C ≤ 800	Esfenvalerate TG (purity not reported), 0, 25, 125, 500, 1000 mg/kg/d, 10/sex/group Dermal exposures were 6 hours per day for 21 consecutive days. A comprehensive FOB and motor activity were included in the clinical assessments.	No mortality. Clinical signs included abnormal hind limb gait in animals at ≥125 mg/kg/d in week 1, vocalisation in females at 500 and 1000 mg/kg/d until day 3, hyperactivity at the start of dosing and then hyper-reactivity at some other times in females at 1000 mg/kg/d. There were no treatment related effects in the FOB. Increased incidences of corneal opacities were reported at ≥125 mg/kg/day, probably due to self-inflicted trauma related to increased scratching rather than to direct systemic toxicity. No toxicologically significant changes in clinical pathology or macroscopic/microscopic pathology. NOAEL 25 mg/kg/d	Anonymous (2000b) RAR B.6.3.4

28 day dietary study in Wistar rats

A non-GLP dietary dose range finding study in Wistar rats is available. Esfenvalerate was given in the diet to Wistar (HanRcc: WIST (SPF)) rats (10/sex/dose) in pelleted diet at a concentration on 0, 300, 500, 700 and 1000 ppm for 4 weeks. Cageside observations were made daily, food consumption and body weights were recorded weekly and, at treatment end, all animals were subjected to necropsy and post mortem examination. Between days 7 and 12 of treatment, 7 males in the top dose group (1000 ppm) 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. At 700 ppm, one male died spontaneously on day 28; the cause of the death could not be determined. No deaths were reported at lower levels.

Excitatory clinical signs such as muscle twitching were present in both sexes at doses ≥ 500 ppm, and abnormal gait and swaying gait was dose-dependently increased in both sexes at doses ≥ 500 ppm. Ataxia was present in all animals at 1000 ppm, and in five females at 700 ppm. Stiff gait was noted in one female at 700 ppm, and

aggressive behaviour/vocalisation when touched was present in females at 1000 ppm. Prostration was noted in one female at the top dose.

Food consumption was dose-dependently reduced in males at doses ≥ 500 ppm and in females at doses ≥ 700 ppm. Mean body weights and body weight gains were dose-dependently reduced in both sexes at doses ≥ 500 ppm. At the end of the treatment period, body weights were reduced by 11.6% in males at 500 ppm, and by 19.7% / 14.0% in males/females at 700 ppm. After one week of treatment, body weights were reduced by 23.6% / 21.3% in males/females at 1000 ppm (animals in this group did not survive long enough to be weighed again).

Treatment-related macroscopic findings were noted at the top dose only, and consisted of dark red discoloured lungs (four males and two females) and dark red discoloured lungs and thymus (one male).

The NOAEL identified in the study report is 300 ppm (equivalent to 22.0 mg/kg bw/d in males and 23.1 mg/kg bw/d in females).

90 day dietary study in Sprague-Dawley rats (1984)

Esfenvalerate was given in the diet to Sprague Dawley derived rats (30/sex/dose), at dose levels of 0, 50, 150, 300 and 500 ppm for up to 13 weeks. 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.

A number of females in the top dose group died or became moribund during the study. Further information on these deaths is provided below:-

Table 31: 90-day dietary feeding study: incidence of mortality[a]

Week of death	♂ (mg/kg bw/d)					♀ (mg/kg bw/d)				
	0	2.5	7.5	15.0	25.0	0	2.5	7.5	15.0	25.0
Total dosed:	30	30	30	30	30	30	30	30	30	30
6	-	-	-	-	-	-	-	-	-	4
7	-	-	-	-	-	-	-	-	-	1
8 (interim necropsy)[b]	10	10	10	10	10	10	10	10	10	9
9	-	-	-	-	-	-	-	-	-	1[c]
11	-	-	-	-	-	-	-	-	-	1
14				1						
14 (at termination)[d]	20	20	20	19	20	20	20	20	20	14
Total no. of animal dying before scheduled necropsy	0	0	0	1	0	0	0	0	0	7

a Data collated from individual survival and sacrifice data

b up to 10 animals/sex killed for interim necropsy

c sacrificed in a moribund state

d up to 20 animals/sex killed for terminal necropsy

A total of 8 rats (7 females in the 500 ppm group and 1 male in the 300 ppm group) died or became moribund during the treatment period. In the 500 ppm group, 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. The incidence of death was not increased along the treatment period. Other than the above, one male in the 300 ppm group died during week 14 immediately after an accidental fall from the cage.

Rats receiving 300 ppm and above exhibited clinical signs such as jerky leg movements and unsteady gait. The severity of these effects was dose related and the high dose group (500 ppm) showed body tremors and became hypersensitive to sounds; some had convulsions and/or death. The signs were usually observed from within the first few weeks of dosing to termination in the high dose group.

Body weight and food consumption decreased significantly in the high dose group and males at 300 ppm. This effect appeared also in other treatment groups early in the study but with time, body weight/food consumption differences between control group and treated group values lessened. There were no consistent haematological changes related to treatment: decreased urine volume and concomitant increase in urine specific gravity were noted in animals having reduced food intake. Decreased absolute mean heart weight and increased relative mean brain weight in the top dose group appeared to be also related to decreased body weight.

The only gross necropsy observation considered to be treatment related was the scab covered areas located at the base of the tail for a few rats fed with 300 and 500 ppm leading to chronic dermatitis. Gross and microscopic evaluation did not reveal any lethal morphologic alteration in the tissues of rats which died during the study.

Microscopic examination revealed after seven and 13 weeks slight to moderate hypertrophy of the parenchymal cells in the parotid salivary gland and with lower incidence in the pituitary glands at the top two dose levels. Gross and microscopic evaluations did not reveal any lethal morphologic alterations in the tissues of the rats found dead during this study.

A NOEL of 50 ppm (equivalent to 2.5 mg/kg bw/d) has been identified in the RAR.

90 day dietary study in Sprague Dawley rats (1987)

This subchronic feeding study was performed in order to evaluate the effect level from the previous 90 day study. Esfenvalerate was admixed in the diet to five groups of Sprague Dawley derived rats (25/sex/dose) at levels of 0, 75, 100, 125 and 300 ppm for either seven (10 rats/sex/group) or 13 (15 rats/sex/group) weeks.

Physical examinations including ophthalmology of all rats were conducted at pre-test and prior to sacrifice. Tissues from the interim rats and terminal rats were preserved but not prepared, processed and examined microscopically, since histopathological evaluation of tissues for the previous study revealed no treatment related morphological changes below 300 ppm.

Treatment related clinical observations were limited to neurological signs in some high dose rats, 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 hindlimbs). This last observation had an overall lack of severity and persistence.

Decreased total weight gain was observed in high dose male rats during the first two weeks of the study only, and in female rats in the 125 and 300 ppm groups throughout the study. There were no significant differences in food consumption between the groups.

Urinalysis, haematology, clinical chemistry and gross necropsy did not reveal treatment related findings. Higher absolute kidney weight was observed in high dose females, and higher relative kidney weight for high dose male and female rats. Relative liver weights were significantly elevated in males receiving 125 or 300ppm group, compared to control males. These differences were not interpreted to be signs of hepatic toxicity because the differences were slight and could be attributed to differences in liver glycogen and/or fat levels or enzymatic induction.

A NOEL of 125ppm (equivalent to 6.25 mg/kg bw/d) has been identified in the RAR, based on neurological clinical signs or kidney weight effects.

Subchronic oral neurotoxicity study in rats (2000c)

Groups of Sprague Dawley rats (12/sex/dose) were fed diet containing 0, 50, 100 or 300 ppm esfenvalerate for 13 weeks. A standard functional observation battery was conducted on study weeks -1, 4, 8 and 13.

Unscheduled deaths, considered to be treatment-related, occurred among males at 300 ppm. Two were killed prior to the scheduled 3 month termination because of the presence of serious skin sores, one on day 52 and the other on day 88.

Adverse effects on body weight, body weight gains and food consumption were observed in males at 100 and 300 ppm, and females at 300 ppm. At termination, body weights were 12.5% lower than controls in males, and 10% lower than controls in females (although the effect in females was not statistically significant).

Treatment-related clinical signs consisted of abnormal gait, which was observed in all males and females at 300 ppm (mean onset days 3/4), which correlated with observations made in the FOB (as described below). Identical observations were made in the earlier esfenvalerate 90-day repeated dose toxicity study at dietary concentrations of 300 ppm and in an esfenvalerate multigeneration reproductive toxicity study (reported in the RAR). Additionally, at 300 ppm there was an increased incidence of skin sores, affecting 6 out of the 12 males; as stated above, for 2 males the wounds were so severe that the animals were killed prematurely. Possibly the skin lesions were related to dermal contact with test substance in the diet, through the use of pelleted diet will have minimised dermal contact.

The key FOB findings consisted of abnormal gait, reductions in forelimb and hindlimb grip strength and a decrease in footsplay. In the open field assessment a number of males and females at 300 ppm were observed with abnormal gait (dragging, hopping) at the 4, 8 and 13 week FOB. Treatment-related reductions in forelimb grip strength were observed in males at 100 and 300 ppm. Among females, forelimb grip strength was statistically significantly reduced in comparison with controls at week 4, but this difference can not be conclusively attributed to treatment as the control value appeared to be unusually high. Treatment-related reductions in hindlimb grip strength were observed at 300 ppm in both males and females. Footsplay was reduced at 300 ppm, only among males.

The motor activity assessment revealed marginal treatment-related effect in males at 300 ppm, only at week 8. The normal pattern of declining motor activity over the 60 minute observation period was less pronounced than observed in the control group, with the number of movements being significantly increased for the 6th 10 minute interval and when analysed as total number of movements over the 60 minute observation period. The duration of movements for males at 300 ppm at week 8 was also increased towards the end of the observation period, although statistical significance was not achieved.

There were no treatment related macroscopic necropsy findings. The microscopic examination of the nervous system tissues did not reveal any treatment-related changes.

In the RAR, NOAELs of 50 ppm (intake of about 3.2 mg/kg/day) for males and 100 ppm (intake of about 7.3 mg/kg/day) for females were identified.

Subchronic dietary neurotoxicity study in Sprague Dawley rats (1999c)

Sprague-Dawley (CD) rats (12/sex/dose) were fed diet containing esfenvalerate at dose levels of 0, 40, 120 or 360 ppm (groups 1 to 4 respectively) for 13 weeks. 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 those 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.

The body weight of the 360 ppm males and females were significantly reduced throughout the treatment period. For males in the 120 ppm group, decreases, occasionally significant, were also observed, while females of this dose group showed values slightly lower than the control group without statistical significance. No significant differences were observed between the control and the 40 ppm groups.

Males and females in the 360 ppm group showed a significant decrease in food intake during the first week of treatment. Other differences noted (a significant decrease for the 120 ppm females from days 50 to 57 and significant increases for the 40 ppm males and females from days 71 to 78 and 78 to 85) were considered to be of no toxicological significance.

At the week 2 FOB, the forelimb grip strength was significantly decreased for males and females in the 360 ppm group. In addition, the ease of removal from the home cage was significantly reduced for the 360 ppm females at this assessment. No significant differences were noted for the subsequent testing occasions.

At the week 2 motor activity assessment, females in the 120 and 360 ppm groups showed a significant ($P<0.05$ or $P<0.01$) decrease in total activity counts when compared to the control and treated groups.

There were no ocular changes considered related to treatment. There were no gross or histopathological changes attributed to treatment. A few males in the 360 ppm groups showed skin ulcerations. There were no significant differences in brain measurements (weight, length or width) between the control and treated groups.

A NOAEL of 40 ppm (intake of 3.0 mg/kg/day) was identified in the RAR.

90 day dietary study in mice

This study was performed to evaluate the subacute toxicity of esfenvalerate and to compare its toxicity with fenvalerate[1]. Groups of B6C3F1 mice 12/sex/dose were fed diets containing 0, 50, 150 or 500 ppm esfenvalerate for 90 days. An additional group of 12 male and 12 female mice was fed diet containing 2000 ppm fenvalerate (in order to compare the two substances, this study was designed so that the diets for both compounds contained the same concentration of the active isomer (Aα)).

The highest dose of esfenvalerate (500 ppm) was equivalent to 106 mg/kg bw/d in males and 113 mg/kg bw/d in females). No deaths occurred in any group. The following clinical signs of toxicity were observed in mice receiving 500 ppm esfenvalerate: 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, and a higher incidence of external lesions such as alopecia, scab and sore formation. The skin lesions were frequently seen in mice exhibiting scratching and licking behavior, and it was considered that the lesions were due to the higher intensity of this behaviour. In relation to the skin lesions, the reactive changes in lymphatic tissues were observed in both sexes as well as increased neutrophil ratio (+125 % in males and +77 % in females receiving 500 ppm esfenvalerate, with no statistical significance) in hematology.

In the animals receiving 500 ppm esfenvalerate, a depression of body weight gain was noted for males (-51 %) and females (-35 %). Water intake was decreased in males and females in the early stage of feeding period but increased thereafter. Effects were also noted on parameters of urinalysis, haematology and clinical chemistry. Effects on urinalysis parameters included significantly lower pH, elevated protein and ketone in males; significantly increased ketones, bilirubin, urobilinogen concentrations and specific gravity in females. The effects on haematology included decreased erythrocyte counts (-11 % in males and -6 % in females), haemoglobin concentration (-11 % in males and -8 % in females) and haematocrit values (-10 % in males and -7 % in females), with slight changes in MCH (-3 %) and MCHC (-2 %) in females. These findings indicate a

[1] Fenvalerate is an insecticide. It is a mixture of four optical isomers, one of which is esfenvalerate. Fenvalerate consists of approximately 23% esfenvalerate.

mild anaemia. Effects on clinical biochemistry included significantly decreased concentration of total protein (-15 % in males and -4% in females), glucose (-31 % in males and -17% in females), cholesterol (-40 % in males and -21 % in females), triglyceride (-84 % in males and -66% in females) and phospholipid (-33 % in males and -32% in females) as well as significant decrease in albumin (-16 %) in males and significant but slight increases in lactate dehydrogenase activity (+41 %), glutamic pyruvic transaminase activity (+69 %) and blood urea nitrogen concentration (+33 %) in females. Besides, leucine aminopeptidase activity was decreased in both sexes (-10 % for both sexes).

At 500 ppm esfenvalerate some changes in organ weights were noted; higher absolute (+15 % in males and +55 % in females) and relative (+37 % in males and +77 % in females) salivary gland weights was observed in both sexes without histopathological changes. A decrease in absolute liver weight (-12 % in males and -9 % in females) was observed and might be related to the decreased body weight gain.

Compound-related histopathological changes in the liver and kidney consisted of decreases in the incidence of diffuse cytoplasmic vacuolation in the liver in both sexes and a decrease in the incidence of cytoplasmic vacuolation in tubular epithelium of kidneys in males. By histochemistry of both tissues (Oil Red O stain), the vacuolations proved to be fatty depositions in the cytoplasm of cells. Other than the above, extramedullary hematopoiesis in the spleen were observed in two females, but the incidences were very low. Limited ulcerative changes such as mucosal erosion, mucosal ulcer and gastritis were observed in the stomachs of 4 males.

Higher liver and spleen weight were observed in mice receiving 2000 ppm fenvalerate and were considered to be related to granulomatous changes observed in the liver and spleen; the absolute liver weight was decreased in mice receiving 500ppm esfenvalerate probably related to the decreased body weight gain; higher salivary gland weight was seen in both esfenvalerate 500 ppm and fenvalerate 2000 ppm groups, but there were no indications of any histopathological change.

Compound-related histopathological changes were observed in the liver, spleen, lymph nodes, thymus, skin, kidney and stomach. They were divided into five categories: 1) microgranulomatous changes in liver, spleen and lymph nodes (only observed in mice receiving 2000 ppm fenvalerate); 2) inflammatory changes in skin (in both high dose compounds); 3) reactive changes in lymphatic tissues (both high dose compounds); 4) ulcerative changes in stomach (slight effect in a few male receiving 500 ppm esfenvalerate); and 5) decrease of fat deposition in liver and kidney (both high dose compounds).

The principal difference between the toxicity of fenvalerate and esfenvalerate was granuloma formation observed in mice receiving fenvalerate at 2000 ppm (microgranulomatous changes were observed in the liver, spleen and lymph nodes). The granuloma formation has been studied with four chiral isomers of fenvalerate using B6C3F1 mice and was considered to be dependent on the content of the Bα-isomer within the test chemicals. In accordance with this conclusion, the microgranulomatous changes were not observed in any tissues of mice treated with esfenvalerate which has a very low content of Bα-isomer (less than 5%).

Reduced leucine aminopeptidase activity was observed only in mice receiving 500 ppm esfenvalerate. Another difference between the two compounds was the ulcerative changes in glandular stomach in the 500 ppm group of esfenvalerate, but with a low toxicological significance.

It was concluded that there were no remarkable toxicological difference between mice fed with esfenvalerate and fenvalerate for three months except the granuloma formation and higher leucine aminopeptidase activity observed only in mice receiving fenvalerate. The NOAEL for esfenvalerate was considered to be 150 ppm for both males and females (intakes of ~30.5 and 36.5 mg/kg/day, respectively).

One Year Oral Study in Dogs

Esfenvalerate was administered to Beagle dogs (6/sex/dose) in the diet over a period of one year at concentrations of 0, 25, 50, 100 and 200 ppm.

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, macroscopic and microscopic findings. Differences noted between treated and control animals in clinical

pathology parameters were considered to be normal biological variations, not important toxicologically, and not related to treatment (inorganic phosphorus, lactate dehydrogenase, total bilirubin and reticulocyte count).

The NOEL was 200 ppm, equivalent to approximately 5 mg/kg/day, the highest dose tested in the study

21 day dermal study in rats

Sprague-Dawley rats (10/sex/dose) were exposed to dermal doses of 0, 25, 125, 500 and 1000 mg/kg bw/day of esfenvalerate for 21 days. Standard investigations (in accordance with the guideline) 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. Treatment-related clinical signs were observed at 125 mg/kg/day and above. During the 1st week, abnormal hind limb gait was observed in all animals in the 500 and 1000 mg/kg/day groups, and in 50% of males and all females in the 125 mg/kg/day group. This observation was consistent with oral toxicity studies conducted with esfenvalerate. Vocalisation was reported for most females at 500 and 1000 mg/kg/day, predominantly during the first 3 days of dosing. Additionally, at 1000 mg/kg/day, 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 application site irritation assessments did not detect any treatment-related local irritation. However, the incidence of probably self-inflicted superficial wounds to the shoulder and forelimb areas was higher in the esfenvalerate-treated groups, likely to be a response to transient local itching and/or tingling sensations which are a known effect of pyrethroids.

The FOB did not reveal any treatment-related effects. However, 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 since motor activity was not increased in females in either the 90-day or acute neurotoxicity studies in rats. Activity, measured as duration of movements, was also increased at 125 mg/kg/day, but this is not considered to be treatment related because the change from the baseline measurement was marginal.

There were no treatment-related adverse effects on bodyweight or food consumption.

The ophthalmoscopy examination revealed increased incidences of corneal opacities at 125 mg/kg/day and above, probably due to self-inflicted trauma related to increased scratching rather that to systemic toxicity.

There were no treatment related haematology or clinical chemistry findings.

There were no organ weight differences, macroscopic or microscopic pathology findings that could be attributed to treatment.

A NOAEL of 25 mg/kg bw/d was identified in the RAR.

Table 32: Summary table of human data on STOT RE

There are no relevant data in humans.

Table 33: Summary table of other studies relevant for STOT RE

There are no other studies relevant for STOT RE.

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

The repeated dose toxicity of esfenvalerate has been investigated by the oral route in rats, mice and dogs, and by the dermal route in rats.

Oral route – rats

Two subchronic toxicity and two repeated dose neurotoxicity studies (all 90 days in duration) have been conducted via the oral route in rats. A 2 year carcinogenicity study is also available, reported in Section 10.9.

In the subchronic and neuorotoxicity studies, significant and severe signs of toxicity were observed at doses relevant for classification (i.e., ≤ 100 mg/kg bw/d). Dose-related neurological effects were observed in all of the studies, and included jerky leg movements, unsteady gait, body tremors, hypersensitivity to sounds, convulsions and reduced grip strength. Neurological effects became apparent at doses ≥ 15 mg/kg bw/d. However, histopathological examinations did not reveal any evidence of significant damage to the nervous system.

In a four week dose range finding study in Wistar rats, all animals (males and females) in the top dose group (44.0 / 46.5 mg/kg bw/d) died or were killed in extremis by day 12 of treatment. One male died at the second highest dose. The cause of death of these animals is not clear. Deaths were also reported in one of the subchronic studies. In this study, conducted in Sprague-Dawley rats, 6 females died and 1 became moribund in the top dose group (25.0 mg/kg bw/d). 4, 1 and 1 females died at weeks 6, 7 and 11 respectively. The moribund female was killed 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 treatment-related deaths were reported in males in this study.

In one of the 90 day studies (Anonymous, 2000c), two males in the top dose group (20.1 mg/kg bw/d) were killed before the end of the study due to the presence of serious skin sores. No other deaths occurred in this study.

No deaths occurred in a 90 day neurotoxicity study which employed a higher top dose (28.8 mg/kg bw/d) than the 90 day study in which deaths were seen. No deaths occurred in a 90 day study with a top dose of 15 mg/kg bw/d.

Treatment-related reductions in food consumption, body weight gain and body weights were reported in more than one study, however specific detail on these effects was generally lacking in the DAR. In one 90 day study, body weights were 12.5% and 10% lower in males and females respectively at the top dose (20.1 mg/kg bw/d) at study termination. In the 2 year study, body weights were reduced in top dose (18.5 mg/kg bw/d) males only (mean body weights were 9.7% lower than controls at study termination).

None of the other toxicological findings seen in the studies are considered to be indicative of significant toxicity in the context of consideration of STOT RE. These included organ weight changes without any histopathological correlates, skin lesions secondary to paraesthesia, and an increased incidence of age-related spinal cord radiculoneuropathy within the historical control range in the 2-year rat study.

Oral route – mice

A 90 day repeated dose toxicity study and an 18 month carcinogenicity study are available in mice via the oral route. No treatment-related effects were observed at doses relevant for classification in the 90 day study. In the carcinogenicity study, all mice at the top dose (18.3 mg/kg bw/d) were sacrificed by day 58 due to excessive self-trauma/mutilation. A large number of mice were also sacrificed early in the other dose group used in the study (4.29 mg/kg bw/d – relevant for classification) due to self-mutilation. The mutilation was considered to be secondary to sensory nerve stimulation following dermal contact with the diet which contained powdered test substance, and not a specific toxic effect following exposure via the oral route.

Oral route - dogs

No treatment-related effects were observed in dogs following oral administration of doses up to 5.02 mg/kg bw/d esfenvalerate for one year.

Dermal route - rats

Dermal exposure to esfenvalerate in the rat at a dose levels ≥ 125 mg/kg/day for 21 days elicited systemic toxicity, observed as abnormal hind limb gait in both genders during the 1st week of treatment. Additionally, increased incidences of corneal opacities were reported at ≥125 mg/kg/day and above, believed to be due to self-inflicted trauma related to increased scratching rather than to direct systemic toxicity. At higher exposure levels, vocalisation, hyperactivity and hyperreactivity were present as a response to esfenvalerate treatment. There were no overt signs of skin irritation at the application sites.

10.12.2 Comparison with the CLP criteria

In mice and dogs, no significant signs of toxicity were observed at doses relevant for classification. Therefore, only the rat data is compared with the CLP criteria for classification purposes.

Rats – Oral Route

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/d (effective dose).

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, it is 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, deaths were observed in a 28 day study (Anonymous, 2008; Wistar rats) and a 90 day study (Anonymous, 1984; Sprague-Dawley rats). In the 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. One male also died at the next highest dose group, on day 28. In the 90 day study, 7 females died in the top dose group (25 mg/kg bw/d). 4, 1, 1 females died after 6, 7 and 11 weeks of treatment, respectively; plus 1 female was found in a moribund condition and killed in week 9. The cause of the deaths was not determined during the study, although convulsions were noted in the animals that subsequently died. In both of these studies, the deaths occurred too late to be considered to be an acute effect. Indeed, 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).

It is not clear why deaths were not seen in other repeated dose studies at similar or even higher doses in the same strain of rats. For example, no deaths or convulsions were observed at the highest doses of 360 ppm (28 mg/kg/d) in a 90-day neurotoxicity study (Anonymous, 1999c) and among adult animals at 350 ppm (22-25 mg/kg/d) in the 2-generation reproduction study (Anonymous, 1994a, reported in the RAR). In addition, no treatment related mortality was observed at 400 ppm in the 2-year study, where the achieved intake during the first 3 months of the study ranged from 32.4 (week 1) to 18.8 (week 13) mg/kg/d for males and 33.4 (week 1) to 22.3 (week 13) mg/kg/d for females (Anonymous, 2011a).

The applicant has commented that the mean achieved intake at 500 ppm in the 90 day study (Anonymous, 1984) has been underestimated, because it was based on a standard conversion factor of 20 (ppm to mg/kg/d). The applicant has calculated the average achieved intake of females at 500 ppm to be 34.2 mg/kg bw/d, using body weight data and food consumption data from the study report, and argues that this is reasonably close to the acute dose levels causing lethality (≥55 mg/kg bw/d). They note that the dead animals in the 90-day study

showed tremors and convulsions prior to their deaths, indicating that the deaths were caused by the neurological effects of esfenvalerate. The applicant argues that the deaths occurred sporadically and that there was no temporal trend in the pattern of mortality that suggests a cumulative effect of repeated dosing; indeed it is well known that lethality from pyrethroid intoxication is attributed to overwhelming acute neurological effects. The applicant concludes that the deaths seen in the repeated dose studies are already covered by the classification for acute toxicity (Acute Tox 3; H301).

However, the dossier submitter notes that deaths occurred up to day 12 in the 28-day study (Anonymous, 2008) and between weeks 6-11 in the 90-day study (Anonymous, 1984). In the opinion of the dossier submitter, these timings are consistent with a repeated dose effect, rather than acute toxicity; therefore classification with STOT-RE is proposed. Deaths occurring at 44.0 / 44.5 mg/kg bw/d in a 28 day study, and at 34 mg/kg bw/d in a 90 day study, support classification in STOT RE Cat 2 ($10 < C \leq 100$ mg/kg bw/d).

Rats – 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. Whilst this dose level is lower than the NOAEL (500 mg/kg bw) in the acute dermal toxicity study in rats, there were no other systemic neurological signs in the 21-day dermal study and this isolated neurological finding is considered not be of sufficient severity to warrant STOT RE classification. Vocalisation, hyperactivity, hyperreactivity and increased motor activity from 500 mg/kg/d were probably secondary to skin sensory stimulation (paraesthesia), rather than due to direct systemic toxicity.

10.12.3 Conclusion on classification and labelling for STOT RE

In a 90 day repeated dose toxicity study via the oral route, deaths occurred in female rats at 25 mg/kg bw/d. On this basis, classification in STOT-RE Cat 2 is proposed.

STOT RE Categroy 2; H373; May cause damage to organs through prolonged or repeated exposure

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**.

10.13 Aspiration hazard

Not evaluated in this report.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Esfenvalerate is an insecticidal active substance considered under Directive 91/414/EEC (subsequently Regulation 1107/2009) for representative use as a foliar insecticide to control a range of insects in spring and winter cereals, potatoes, and spring and winter oilseed rape. Available environmental fate and ecotoxicology studies have been considered and summarised in the revised Renewal Assessment Report, June 2014 (volume 3, annex B.8: Environmental fate and behaviour and volume 3, annex B.9: Ecotoxicology). The outcome of the pesticide peer review and agreed endpoints from this process are summarised in the EFSA conclusion (EFSA Journal 2104;12(11):3873).

The key information pertinent to determining the environmental hazard classification for esfenvalerate is presented below. Unless otherwise stated, these studies were conducted in accordance with GLP and the validity criteria of the respective test guideline, if applicable. Full robust summaries of these studies are presented in Annex 1 to this dossier. Further information on the parent and environmental degradates are presented in Annex 3 of this dossier.

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 the original evaluation, however they are largely supporting information and none of the critical endpoints relate to fenvalerate.

Esfenvalerate is hydrolytically stable under acidic conditions, but degrades under neutral and alkaline conditions to primary degradates, with low volatilisation. Degradation was also observed under photolytic conditions to primary degradates, with low volatilisation. Esfenvalerate was found to not be readily biodegradable. Degradation was observed in natural water/sediment systems, mainly in the sediment phase, to primary degradates. Degradation of esfenvalerate under photolytic conditions was calculated using the GCSOLAR programme as < 1.4 days.

Under hydrolytic conditions, esfenvalerate was rapidly converted to its 2*S*α*R*-isomer under neutral and alkaline conditions to a 1:1 ratio, and remained at almost the same ratio for the entire incubation period at all tested temperatures. However, no isomerisation was observed under acidic and/or photolytic conditions.

11.1 Rapid degradability of organic substances

Table 34: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
Aquatic hydrolysis, performed according to: OECD guideline 111 (April 2004)	pH 4: stable. pH 7: 427.7 days at 20°C (1st order). pH 9: 5.3 days at 20°C (1st order).	Valid – study performed to GLP. All calculated degradation rates are for the sum of esfenvalerate and the 2*S*α*R* isomer. Test substance: [Phenoxyphenyl-^{14}C]-esfenvalerate (purity: 99.3%, optical purity 98.7%) [Chlorophenyl-^{14}C]-esfenvalerate (purity: 99.8%, optical purity 99.1%)	Graham R., Gilbert J. (2012) LLM-0068 RAR B.8.4.1

Method	Results	Remarks	Reference
Aquatic photolysis, performed according to: OECD guideline 316 (October 2008)	DT_{50}: 2 days (Chi^2 error = 9.51) 5.5% volatilisation at 30 days. Quantum yield for esfenvalerate was determined to be 0.016 at Σ >290 nm.	Valid – study performed to GLP. Xenon lamp, 50°N, equivalent to UK/US summer sunlight. Esfenvalerate was not isomerised under irradiation. Dark controls showed no significant degradation. Test substance: [Phenoxyphenyl-^{14}C]-esfenvalerate (specific activity 10.84 MBq/mg, radiochemical purity 99.3%) [Chlorophenyl-^{14}C]-esfenvalerate (specific activity 10.22 MBq/mg, radiochemical purity 99.8%)	Graham R., Dove R. (2012) LLM-0065 RAR B.8.4.2
Esfenvalerate: Calculation of aqueous photolysis rates in near surface water at north latitudes 10º and 80º using GCSOLAR programme	The calculated photolytic half-lives of esfenvalerate were 1.28 to 1.36 days at latitudes of 30, 40 and 50ºN in summer.	Not GLP – calculation only.	Suzuki Y., T. Fujisawa T., Katagi T. (2012) LLM-0103 RAR B.2.9.2
Ready biodegradation screening, performed according to: OECD guideline 301B (revised 1992)	Esfenvalerate is considered not ready biodegradable, with 0% degraded by 28 days.	Valid – study performed to GLP. Performed at 22 ± 2°C. Test substance purity stated to be 100% esfenvalerate.	Graham R., Flenley A. (2011) LLM-0058 RAR B.8.4.3
Degradation and fate in simulated water/sediment systems for up to 100 days, performed according to: UK guideline for conduct of biodegradability tests on pesticides in natural sediment-water systems, kinetic modelling subsequently performed according to FOCUS guidance (2006)	DT_{50} = 25.3 to 30.7 days (total system, normalised to 20°C). 3.2% to 5.2% mineralisation after 100 days. DT_{90} = 216.9 to 263.3 days (total system at 10°C).	Valid – study performed to GLP. Test substance: [Chlorophenyl-^{14}C]-esfenvalerate (>97.5% radiochemical purity, 164 µCi/mg specific activity)	Lewis C. (1995) LLM-0040 and Jarvis T., Mamouni A. (2011c) LLM-0059 RAR B.8.4.4

11.1.1 Ready biodegradability

A new study (Graham and Fenley, 2011, LLM-0058) was submitted on the ready biodegradability of esfenvalerate during the EU review. The study followed the OECD guideline 301b (revised 1992) and was conducted to GLP. UK activated sewage sludge was collected from a sewage treatment works and added to a buffered mineral medium. Esfenvalerate was then added to give a nominal concentration of 15 mg carbon/L.

Five additional vessels were also prepared; two reference substance control vessels containing sodium benzoate, two blank control vessels containing only the buffer medium and a single toxicity control vessel containing both the esfenvalerate and sodium benzoate.

The test system was incubated at 22 ± 2ºC in the dark. Trap analysis for evolved CO_2 was performed at regular intervals (10 sample intervals in total) for up to 28 days after treatment. The theoretical yield of CO_2 from the vessels was calculated and the cumulative values for the test substance, reference substance and toxicity control vessels were corrected against the blank controls.

The theoretical yield of evolved CO_2 from esfenvalerate was 0% at 28 days. Hence, esfenvalerate was considered as being classified as 'not readily biodegradable'.

11.1.2 BOD_5/COD

No information submitted.

11.1.3 Hydrolysis

A new study (Graham and Gilbert, 2012, LLM-0068) was submitted on the hydrolysis of esfenvalerate in buffered solutions for the EU review. The study followed the OECD guideline 111 (April 2004) and was conducted to GLP. A summery is provided below, with a robust summary provided in the Annex 1 to dossier.

A tier I test was performed at 50ºC with sterile buffer solutions at pH 4, pH 7 and pH 9 using two radiolabelled forms of esfenvalerate. Esfenvalerate was found to be hydrolytically stable at pH 4, however at pH 7 and pH 9, > 10% hydrolysis occurred after five days, triggering a tier II aqueous hydrolysis test for pH 7and pH 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.

Mean mass balances were in the range 88.9 – 104.2% AR (applied radioactivity) for all sampling points and all tier II incubation temperatures at pH 4, 7 and 9. Sterility and pH (within ± 2) of the samples were maintained during the study period.

Two major hydrolytic degradates were CPIA and PBald, which were formed via ester cleavage of esfenvalerate. 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 degradate $CONH_2$-Fen exceeding 10% AR. The maximum levels observed were in the range of 10.1 to 11.1% AR. Under alkaline conditions, $CONH_2$-Fen further degraded to 3-phenoxymandelic acid and CPIA-carboxamide across all three temperature ranges. The degradate 3-Phenoxymandelic acid was observed at maximum levels in the range of 8.6% to 11.3% AR and CPIA-carboxamide was observed at maximum levels in the range of 10.2% to 12.2% AR. All other degradates including PBacid and PBCN accounted for less than 10% AR throughout the study.

Chiral analysis of samples also showed that esfenvalerate was rapidly converted to its 2*S*α*R*-isomer under neutral and alkaline conditions to a 1:1 ratio, and remained at almost the same ratio for the entire incubation period at all tested temperatures. All calculated degradation rates are for the sum of esfenvalerate and the 2*S*α*R* isomer.

The activation energy values for hydrolysis at pH 7 and 9 were calculated to be 98.6 kJ/mol and 103.3 kJ/mol, respectively. 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.

11.1.4 Other convincing scientific evidence

No information provided.

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

No information provided.

11.1.4.2 Inherent and enhanced ready biodegradability tests

No information provided.

11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

Two water sediment studies (Takahashi and Oshima, 1988, LLM-0024 and Lewis, 1995, LLM-0040) were submitted on the degradation of esfenvalerate in water - sediment systems during the EU review. One study (Takahashi and Oshima, 1988, LLM-0024) was not conducted to any known guidelines and pre-dated GLP. As this study does not meet current data requirements, is has not been summarised in this dossier. The second study (Lewis, 1995) was conducted to the proposed UK Guidelines for the conduct of biodegradability tests on pesticides in natural sediment - water systems and was conducted to GLP. A kinetic assessment (Jarvis and Mamouni, 2011, LLM-0059) was performed in accordance FOCUS (2006) guidance on the data generated from both studies and submitted during the EU review. Only the data from the Lewis (1995, LLM-0040) has been reported in this dossier. Both studies were considered acceptable during the EU review.

Esfenvalerate was observed to dissipate rapidly to the sediment layer and degrade in water-sediment systems to several degradation products.

Summaries of the two studies are presented below, with robust summaries presented in Annex 1 of this dossier.

Study 1 (Lewis, 1995, LLM-0040)

The degradation of esfenvalerate was investigated in two aquatic systems using one radiolabelled form of esfenvalerate. The systems were set up with natural sediment and associated waters, and CO_2 free air was bubbled gently into the water layer. Suitable traps for collecting volatile compounds were connected to the system. The systems were equilibrated for up to 76 days, before esfenvalerate was applied to the water layer. Samples were incubated for up to 100 days at 10ºC in the dark.

At nine appropriate time points, duplicate flasks were taken and the water and sediment separated. The water layer was partitioned with dichloromethane concentrated to dryness and re-constituted in methanol. The sediment was extracted with acetone, and then extracted by Soxhlet. The resulting extract was acidified, partitioned with dichloromethane, then concentrated to dryness and re-constituted in methanol. Radioactivities in the aqueous and combined organic phase from surface water as well as the radioactivity from the sediment were determined by LSC. Evolved volatile radiolabelled material was also collected for analysis at the same sampling intervals and the radioactivity was determined by LSC. In both systems esfenvalerate and its degradation products were determined by TLC and HPLC.

Recoveries in the two systems were 80.74% to 98.51% of applied radioactivity. The lower recoveries at 0.25 to 14 days were attributed to loss of radioactivity on dip tubes and probes.

In two water/sediment systems, esfenvalerate dissipated very rapidly from the water phase (first order DT_{50} 5.3 to 8.9 days, not taking into consideration that >50% esfenvalerate had already partitioned into prior to the t=0 sampling point)). 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). A number of minor degradation products were also produced but in relatively small amounts. Low levels of volatile radioactivity were evolved, one of the components probably being carbon dioxide. Low levels of bound residues were formed.

Study 2 (Jarvis and Mamouni, 2011, LLM-0059)

A kinetic re-evaluation of the water sediment studies was undertaken (Takahashi and Oshima, 1988, LLM-0024 and Lewis, 1995, LLM-0040) in accordance with FOCUS kinetics guidance (2006). The first study

(Takahashi & Oshima, 1988, LLM-0024) was not performed to current guidance and does not meet the data requirements. Therefore the results from this study have not been summarised.

The assessment on visual and statistical fit, residual plot and χ^2 error was undertaken using the recommendations of FOCUS kinetics guidance (2006). Kinetic modelling was performed using KinGUI version 2.0.

In the overall water/sediment systems at 10°C the first order DT_{50} and DT_{90} values ranged from 65.3 to 79.3 days, and 216.9 to 263.3 days, respectively, demonstrating significant degradation even in the sediment phase. The DT_{50} values (normalised to 20ºC) ranged from 25.3 to 30.7 days.

11.1.4.4 Photochemical degradation

Two new studies (Graham and Dove, 2012, LLM-0065 and Suzuki, Fujisawa and Katagi, 2012, LLM-0103) were submitted on the aqueous photolysis of esfenvalerate in buffered solutions during the EU review. One study followed the OECD guideline 316 (October 2008) and was conducted to GLP (Graham and Dove, 2012). The second study (Suzuki, Fujisawa and Katagi, 2012, LLM-0103) was a calculation based on the results from the first study, therefore GLP does not apply. Summaries of the studies are provided below with robust summaries provided in the Annex 1 of this dossier.

Study 1 (Graham and Dove, 2012, LLM-0065)

The aqueous photolysis of esfenvalerate in sterile aqueous buffer at pH 4 ± 2 was investigated using two radiolabelled forms of esfenvalerate. Esfenvalerate was applied under sterile conditions to a sodium acetate buffer (with acetonitrile as a co-solvent) and vessels were sealed with quartz lids. Dark control vessels were dispensed into glass jars. All vessels contained polyurethane bungs in the inlet/outlet arms, attached to bacterial air filters, and a security trap, followed by two sodium hydroxide traps for volatiles. Samples were irradiated for up to 30 days at 25 ± 2°C, using UV filtered light with the average intensity being adjusted to *ca* 25 W/m^2 over the 300 – 400 nm range, so that light received within 30 days was equivalent to 30 days of UK/US summer sunlight. Dark controls were maintained at 25 ± 2°C in the dark. A PNAP/PYR actinometer was also included for the determination of quantum yield.

Sampling occurred in duplicate at 0, 1, 3, 7, 14, 21 and 30 DAT. On analysis, samples were acidified to <pH 2, partitioned with dichloromethane and the organic layer concentrated. Volatiles were analysed using the polyurethane foam bungs and sodium hydroxide traps. A vessel rinse was also performed after removal of the buffer. All samples were quantified by LSC and the concentrated organic extract analysed by HPLC. Chiral HPLC analysis was also used to investigate possible fenvalerate isomers.

Mean mass balances for each sampling point ranged from 96.2% to 103.8%. Radioactivity was found mainly in the organic layer. In the irradiated samples, radioactivity decreased in the organic layer to 78.5% to 79.0% AR, with the aqueous layer increasing to 13.0% to 13.4% AR by the end of the study period. Radioactivity in the dark controls stayed in the organic layer. Little radioactivity was detected in the vessel rinses, with maximum levels observed at <1% AR. Volatilisation was low, with maximum levels at 5.5% AR by 30 DAT.

Esfenvalerate was seen to degrade under photolytic conditions to <1.7% AR by 21 DAT. The major degradates observed were PBacid, Dec-fen A, Dec-fen B and PA-Fen, with the mean maximum levels >10% AR. The degradates 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.

Chiral analysis of selected samples showed that esfenvalerate was not isomerised under irradiation.

The SFO 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 quantum yield for esfenvalerate was determined to be 0.016.

Study 2 (Suzuki, Fujisawa and Katagi, 2012, LLM-0103)

The aqueous photolysis rate of esfenvalerate in near surface water at northern latitudes of 10 to 80ºN were calculated using the GCSOLAR (ver. 1.2) programme. The half-lives were calculated as a function of season, latitude, time of day, depth of water bodies and ozone layer thickness. The input data were obtained from the report on photodegradation and quantum yield in sterile, aqueous solution of esfenvalerate (Graham and Dove, 2012).

11.2 Environmental transformation of metals or inorganic metals compounds

Not applicable.

11.2.1 Summary of data/information on environmental transformation

Not applicable.

11.3 Environmental fate and other relevant information

11.3.1 Volatilisation

Esfenvalerate has a vapour pressure of 1.17 x 10^{-9} Pa (Wells, 1998, LLP-0074) and water solubility of 0.001 mg/L (Kogovsek, 1997), both at 20°C, resulting in a Henry's law constant of 4.92 x 10^{-4} $Pa.m^3.mol^{-1}$ (Yoshimura, 1998, LLP-0078). This combination of properties indicates that volatilisation from aqueous systems / soil water is likely to be low.

The reaction of esfenvalerate in the atmosphere with hydroxyl radicals was estimated using the Atkinson's calculation (calculated by the RMS). The atmospheric half-life of esfenvalerate was estimated to be 0.48 days assuming an OH radical concentration of 1.5 x 10^6 radicals cm^{-3} for a 12 hour day.

Therefore, esfenvalerate would be unlikely to be subject to long range aerial transport and air is not a likely route of environmental contamination.

11.4 Bioaccumulation

Table 35: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
Partition coefficient *n*-octanol/water; Esfenvalerate AS (purity 99.4%); OECD 107	log P_{OW} = 6.24 at 25°C (pH not stated)	Valid (accepted endpoint from EU RoA evaluation)	Tanoue A., Itoh K. (1989); LLP-0033; RAR B.2.1.13 (i)
Partition coefficient *n*-octanol/water; Esfenvalerate AS (purity 99.9%); OECD 107	log P_{OW} = 5.0 at 23°C (pH 7.3)	Valid	Rohr G. (1991); LLP-0043; RAR B.2.1.13 (ii)
Experimental aquatic BCF test in fish to US EPA Guideline 165-4; non-GLP; purity (both labels) 99.4% (radiochemical), >99% (chemical)	Esfenvalerate steady state whole fish BCF (TRR and esfenvalerate): 2850 and 3110 L/kg wet weight [^{14}C-chlorophenyl] esfenvalerate; 3340 and 3650 L/kg wet weight [^{14}C-phenoxyphenyl] esfenvalerate Depuration half-life (DT_{50} whole fish (TRR and esfenvalerate): 6.50 and 7.88	Study on *Cyprinus carpio*. Flow through, 28 days exposure, 14 days depuration Analysis of total radioactive residue and parent esfenvalerate	Anonymous (1991); LLM-10-0031; RAR B.9.2.3 (i)

Method	Results	Remarks	Reference
	days [^{14}C-chlorophenyl] esfenvalerate; 6.89 and 7.80 days [^{14}C-phenoxyphenyl] esfenvalerate	Valid (BCF of 3110 used in RoA List of Endpoints)	
Experimental aquatic BCF test in fish to US EPA Guideline 165-4; non-GLP; purity (all labels) >99% (radiochemical)	S-fenvalerate whole fish BCF (TRR and S-fenvalerate) 1537-1844 and 1245-1494 L/kg wet weight; Depuration half-life (DT_{50} whole fish: ca. 5 days Aquatic model ecosystem BCF (TRR and S-fenvalerate): fish 174-257 and 69-117; Daphnia 191-234 and 269-322; snail 428-472 and 386-491; algae 283-515 and 278-506	Semi-static, 7 days exposure, 28 days depuration (fish); static, 7 and 30 days exposure (aquatic model ecosystem containing sediment) Analysis of total radioactive residue and S-fenvalerate Valid (supporting information only)	Ohkawa H., Kikuchi R., Miyamoto J. (1980); AM-00-0108; RAR B.9.2.3 (i)

11.4.1 Estimated bioaccumulation

As experimental data are available, estimations of bioaccumulation potential are not required.

11.4.2 Measured partition coefficient and bioaccumulation test data

The Log K_{OW} of esfenvalerate is 6.24, this is greater than the trigger in the CLP Regulation of ≥4 which could influence the chronic aquatic classification for esfenvalerate, particularly if adequate chronic data were not available. However, a reliable experimental fish bioconcentration factor (BCF) for Common carp (*Cyprinus carpio*) is available from the study by Anonymous (1991, LLM-10-0031). 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. Esfenvalerate accounted for 40 - 75% of the total radioactive residues in the whole fish but none of the metabolites identified were present in the whole fish at levels of 0.05 ppm or greater. There was no growth or lipid correction but this is not considered to substantively affect the results, or the conclusion regarding bioaccumulation potential.

In a second study (Ohkawa, Kikuchi & Miyamoto, 1980, AM-00-0108) submitted as supporting information only, carp were exposed to ^{14}CN-S-fenvalerate for 7 days. Two model ecosystem studies in a semi-field design (water-sediment system) were carried out in which carp as well as *Daphnia* and field collected snails were added. These studies indicated that (*S*)-fenvalerate (esfenvalerate and its [2*S*, α*R*] isomer) is rapidly taken up, extensively metabolised and readily eliminated by carp. This is however of uncertain relevance in relation to hazard classification.

The maximum fish BCF values for esfenvalerate (esfenvalerate and total radioactivity) are greater than the trigger of ≥500 in the CLP regulation requiring consideration of the impact of bioconcentration on its chronic classification. However, since esfenvalerate is already considered 'not rapidly degradable' and adequate chronic data are available, this would not in any case affect the decision on chronic classification and M-factor.

11.5 Acute aquatic hazard

Table 36: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results	Remarks	Reference
Guideline not specified	Bluegill sunfish (*Lepomis macrochirus)*	Esfenvalerate, purity not specified	LC_{50}= 0.21 µg/L (95% confidence limits; 0.18-0.28), based on nominal concentrations. Measured concentrations were between 80 and 105% of nominals.	Flow-through system, 96 hours exposure. Not GLP compliant.	Anonymous (1985k); LLW-50-0004; RAR B.9.2.1 (i)
Guideline EPA 660/3-75-009 (1975)	Rainbow trout (*Salmo gairdneri)*	Esfenvalerate, purity 98.8%	LC_{50}= 0.26 µg/L (95% confidence limits; 0.20-0.38), based on nominal concentrations. Actual test concentrations were not monitored. Therefore, nominal concentrations are potentially unreliable.	Static-renewal system, 96 hours exposure. GLP compliant. No information on illumination during the exposure period.	Anonymous (1985l); LLW-51-0010; RAR B.9.2.1 (ii)
Guideline OECD 203 (1981)	Rainbow trout (*Salmo gairdneri)*	Esfenvalerate, purity 94.5%	LC_{50}= 0.10 µg/L (95% confidence limits; 0.05-0.17), based on nominal concentrations. Measured concentrations were between 107 and 125% at the nominal values of 0.032 and 0.056 µg/L. Therefore, expression of the endpoint in terms of nominal concentrations is potentially unreliable.	Flow-through system, 96 hours exposure. Not GLP compliant. No details of the light dark cycle were given.	Anonymous (1986f); LLW-60-0009; RAR B.9.2.1 (iii)

Method	Species	Test material	Results	Remarks	Reference
Guideline EPA 660/3-75-009 (1975)	Fathead minnow (*Pimephales promelas)*	Esfenvalerate, purity 98.8%	LC_{50}= 0.18 µg/L (95% confidence limits; 0.13-0.36), based on nominal concentrations. Measured concentrations at test initiation and after two days were 85 and 50% of the nominal value of 1.0 µg/L respectively. Therefore, nominal concentrations are potentially unreliable. All fish at 1.0 µg/L were dead by the time the second measurement was taken (after two days) however and therefore, this later drop in concentration is not considered to have substantially affected the results based on nominals.	Static-renewal system, 96 hours exposure. Not GLP compliant. Toxicity symptoms were not fully reported.	Anonymous (1984b); LLW-41-0021; RAR B.9.2.1 (iv)
Guideline OECD 202 (1984)	Water flea (*Daphnia magna)*	Esfenvalerate, purity 86.6%	EC_{50}= 27 µg/L (95% confidence limits; 21-36), based on mean measured concentrations.	Static-renewal system, 48 hours exposure. GLP compliant.	Sayers L. E. (2005); LLW-0120; RAR B.9.2.1 (xi)
Guideline US EPA 72-2 (1985)	Water flea (*Daphnia magna)*	Esfenvalerate, purity 98.6%	EC_{50}= 0.9 µg/L (95% confidence limits; 0.7-1.16), based on nominal concentrations. Actual concentrations were not measured, therefore, nominal concentrations are potentially unreliable.	Static-renewal system, 48 hours exposure. GLP compliant.	Hutton D. G. (1987); LLW-71-0029; RAR B.9.2.1 (xiii)
Guideline US EPA 72-2 (1985)	Water flea (*Daphnia magna)*	Esfenvalerate, purity 98.6%	EC_{50}= 3.5 µg/L (95% confidence limits; 2.7-4.9), based on nominal concentrations. Actual concentrations were not measured, therefore, nominal concentrations are potentially unreliable.	Static-renewal system, 48 hours exposure, feeding, GLP compliant.	Hutton D. G. (1987); LLW-71-0028; RAR B.9.2.1 (xiv)

Method	Species	Test material	Results	Remarks	Reference
Guideline OECD 202 (2004)	Water flea (*Daphnia magna)*	Esfenvalerate Aβ isomer, purity 98.8% and esfenvalerate technical, purity 87.3%	EC_{50}= 0.21 µg/L (95% confidence limits; 0.10-0.31), based on mean measured concentrations. EC_{50} <0.049 µg/L based on mean measured concentrations (55% immobilisation seen at this conc.n, so actual EC_{50} assumed to be approx. 0.045 µg/L).	Static-renewal system, 48 hours exposure. GLP compliant.	Sayers L. E. (2011); LLW-0142; RAR B.9.2.1 (xii)
Guideline OECD 201 (1984)	Green algae (*Scenedesmus subspicatus)*	Esfenvalerate, purity 97%	E_bC_{50} (96 hr)= 6.5 µg/L and E_rC_{50} (24-48 hr)= 10.0 µg/L, based on initial nominal concentrations. Mean measured concentrations ranged from 123 to 136% of nominal concentrations at 0 hours and 113.8 to 171% after 96 hours. Therefore, nominal concentrations are potentially unreliable.	Static-renewal system, 96 hours exposure. GLP compliant.	Handley J. W., Sewell I. G., Bartlett A. J. (1991); LLW-01-0038; RAR B.9.2.1 (xxi)

* Whilst some of the earlier (1980s) studies were not GLP compliant, and some guideline or reporting deviations were observed, studies are overall considered to be sufficiently reliable and appropriate for use in hazard classification. The endpoints based on nominal concentrations, where measured values deviated from 80-120% of nominals, should however be used with some caution (discussed below).

11.5.1 Acute (short-term) toxicity to fish

Four studies have been submitted on the acute toxicity of esfenvalerate to fish. Two of these were carried out with rainbow trout (*Oncorhynchus mykiss*), one with bluegill sunfish (*Lepomis macrochirus*), and one with fathead minnow (*Pimephales promelas*). The fathead minnow study and the rainbow trout study by Anonymous (1985l, LLW-51-0010) were undertaken following the US EPA 660/3-75-009 (1975) guideline, but only the latter was performed according to GLP standards. The guideline used for the test with bluegill sunfish was not specified in the study report. The rainbow trout study by Anonymous (1986f, LLW-60-0009) was performed according to the OECD 203 (1981) guideline and the resulting LC_{50} value of 0.10 µg/L was the lowest among the four studies. Therefore, rainbow trout was identified as the most sensitive fish species. This endpoint was based on nominal concentrations; measured concentrations were 107-125% of nominals and so close to, but exceeding, 80-120%. A measured endpoint was not presented but would be slightly >0.10 µg/L. This trout endpoint should therefore be treated with some caution but is considered sufficiently precautionary for hazard classification purposes. Further details of these studies can be found in Annex 1 Section 4.3.1 of this CLH report.

Four studies have been submitted under Regulation (EC) No 1107/2009 on the acute toxicity of the esfenvalerate metabolites 3-phenoxybenzoic acid, Dec-Fen, $CONH_2$-Fen and PA-Fen to rainbow trout (*Oncorhynchus mykiss*). These studies were undertaken according to relevant OECD 203 and to GLP and are considered reliable. In addition, an acute toxicity study with killifish (*Oryzias latipes*) has been submitted for (+)CPIA and although this was not considered acceptable in the RAR, the result is considered indicative of the

toxicity of this metabolite to fish. They reported acute 96 hr LC_{50} values for 3-phenoxybenzoic acid, Dec-Fen, (+)CPIA, $CONH_2$-Fen and PA-Fen were 14.3, >0.99, 74.1, 0.11 and >0.703 mg/L, respectively (see Table 1, Appendix 2). It is appropriate that these were conducted on the same most acutely sensitive fish species tested with the parent compound (except in the case of CPIA(+)). The data indicate that all the metabolites are much less toxic to fish than the parent esfenvalerate (by at least two orders of magnitude) and so are not considered further for classification of esfenvalerate. As they are considered supporting data, they have not been reported here in detail but they are evaluated in full in section B.9.2.1 of the esfenvalerate RAR.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

Four studies have been submitted on the acute toxicity of technical esfenvalerate to *Daphnia magna*. Two of these studies were undertaken following the US EPA 72-2 (1985) guideline, and the other two followed OECD 202 (1984 and 2004). All four studies were conducted according to GLP. The reported 48-hour EC_{50} values for *D. magna* were 27, 0.9, 3.5 and <0.049 µg/L; as 55% immobilisation was seen at this last concentration (study by Sayers, 2011) the actual EC_{50} is assumed to be approximately 0.045 µg/L. In addition an EC_{50} value of 0.21 µg/L resulted from testing *D. magna* with esfenvalerate 2SαR-isomer (Aβ isomer) - also in Sayers (2011). This comparative study gave the lowest endpoint for technical esfenvalerate under similar test conditions, indicating that it was more toxic than the 2SαR-isomer. There is no evidence that it would occur but if full epimerization of esfenvalerate to this isomer were to occur in surface waters, the potential hazard to aquatic organisms would therefore be addressed by the assessment for technical esfenvalerate and this would not affect its classification. Further details of these studies can be found in Annex 1 Section 4.3.2 of this CLH report.

Since there are ≥ four reliable endpoints for the same species (*D. magna*) then according to the ECHA Guidance on the Application of the CLP Criteria (2017) a geometric mean may be taken of these endpoints for technical esfenvalerate (i.e. 27, 0.9, 3.5 and ≈ 0.045 µg/L). This results in a geomean value for the acute toxicity to aquatic invertebrates of 1.4 µg/L.

Five studies have been submitted under Regulation (EC) No 1107/2009 on the acute toxicity of the esfenvalerate metabolites 3-phenoxybenzoic acid, Dec-Fen, $CONH_2$-Fen and PA-Fen to *Daphnia magna*. These studies were undertaken according to OECD 202 and to GLP and are considered reliable. The reported acute 48 hr EC_{50} values for 3-phenoxybenzoic acid, Dec-Fen, (+)CPIA, $CONH_2$-Fen and PA-Fen were 35.4, >0.86, 74.0, >0.93 and >0.382 mg/L, respectively (see Table 1, Appendix 2). The data indicate that all the metabolites are much less toxic to *Daphnia* than the parent esfenvalerate (by at least an order of magnitude) and so are not considered further for classification of esfenvalerate. They are, therefore, considered supporting data and have not been reported here in detail but they are evaluated in full in section B.9.2.1 of the esfenvalerate RAR.

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

A study has been submitted on the toxicity of esfenvalerate to green algae (*Scenedesmus subspicatus*). This study was undertaken following the OECD 201 (1984) guideline, and the resulting 96-hour E_bC_{50} was 6.5 µg/L and E_rC_{50} value was 10.0 µg/L based on nominal concentrations. Further details of these studies can be found in Annex 1 Section 4.3.3 of this CLH report.

Five studies have been submitted under Regulation (EC) No 1107/2009 on the acute toxicity of the esfenvalerate metabolites 3-phenoxybenzoic acid, Dec-Fen, $CONH_2$-Fen and PA-Fen to *Pseudokirchneriella subcapitata*. These studies were undertaken according to OECD 201 and to GLP and are considered reliable. The reported 72 hr E_bC_{50} values for 3-phenoxybenzoic acid, Dec-Fen, (+)CPIA, $CONH_2$-Fen and PA-Fen were 33.8, >0.24, 64.6, >0.15 and >0.421 mg/L, respectively and the 72 hr E_bC_{50} values were 51.92, >0.24, >100, >0.15 and >0.421 mg/L, respectively (see Table 1, Appendix 2). The data indicate that all the metabolites are much less toxic to algae than the parent esfenvalerate (by at least an order of magnitude) and so are not considered further for classification of esfenvalerate. They are, therefore, considered supporting data and have not been reported in detail here but they are evaluated in full in section B.9.2.1 of the esfenvalerate RAR.

11.5.4 Acute (short-term) toxicity to other aquatic organisms

No acute toxicity data are available for other aquatic organisms.

11.6 Long-term aquatic hazard

Table 37: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results	Remarks*	Reference
Guideline OECD 204 (1984)	Rainbow trout (*Salmo gairdneri)*	Esfenvalerate, purity 97%	NOEC= 0.001 µg/L, based on nominal concentrations. Mean measured stock solution concentrations were between 121 and 123% of nominals, there did not appear to be actual measurement of exposure in test media. The nominal endpoint is therefore potentially unreliable.	Flow-through system, 21 days exposure. GLP compliant.*#	Anonymous (1991b); LLW-01-0036; RAR B.9.2.2 (i)
Guideline US EPA (1971) 'Recommended bioassay procedure for fathead minnows (*Pimephales promelas*, Rafinesque) chronic tests'	Fathead minnow (*Pimephales promelas)*	Fenvalerate, purity 96% (includes esfenvalerate isomers)	Maximum acceptable toxicant concentration >0.090 and <0.21 µg/L, and NOEC= 0.090 µg/L, based on mean measured concentrations.	Flow-through system, 260 days exposure (full life cycle). Not GLP compliant.	Anonymous (1978b); AW-81-0071; RAR B.9.2.2 (ii)
Guideline US EPA 72-4 (1985)	Water flea (*Daphnia magna)*	Esfenvalerate, purity 98.6%	Maximum acceptable toxicant concentration >0.052 and <0.079 µg/L, and NOEC= 0.052 µg/L, based on mean measured concentrations.	Semi-static system, 21 days exposure. GLP compliant.	Hutton D. G. (1987); LLW-71-0027; RAR B.9.2.2 (iv)

Method	Species	Test material	Results	Remarks*	Reference
Guideline OECD 202 Part II (1985)	Water flea (*Daphnia magna)*	Esfenvalerate, purity 97%	NOEC= 0.0018 µg/L, based on nominal concentrations. Mean measured concentrations in the solvent stock solutions were between 99 and 110% of nominal values. Actual exposure concentrations in test media were not measured however, therefore nominal concentrations are potentially unreliable.	Semi-static system, 21 days exposure. GLP compliant.	Handley J. W., Sewell I. G., Barlett A. J. (1991); LLW-01-0037; RAR B.9.2.2 (v)
Guideline BBA (1995)	Non-biting midge (*Chironomus riparius*)	[^{14}C] Esfenvalerate , purity 98.9%	NOEC= 0.160 µg/L, based on nominal concentrations added to water phase. At test termination, recoveries in the water phase were between 3 and 6% of nominal concentrations. A mean measured endpoint has not been quoted, therefore the NOEC has only be expressed in terms of initial nominals in the water phase.	Static system containing sediment, 28 days exposure. Spiked water study. GLP compliant. Used 10% peat, whereas 4-5% peat is stipulated in the guideline. Organic carbon content was also lower than recommended at 1.4%.	Putt A. E. (1997); LLW-0085; RAR B.9.2.2 (vii)
Guideline OECD 201 (1984)	Green algae (*Scenedesmus subspicatus*)	Esfenvalerate, purity 97%	96 hr NOEC (growth rate and biomass) = 1.0 µg/L based on nominal concentrations. Mean measured concentrations ranged from 123 to 136% of nominal concentrations at 0 hours and 113.8 to 171% after 96 hours. Therefore, nominal concentrations are potentially unreliable.	Static-renewal system, 96 hours exposure. GLP compliant.	Handley J. W., Sewell I. G., Bartlett A. J. (1991); LLW-01-0038; RAR B.9.2.1 (xxi)

* Whilst one of the earlier studies (Anonymous, 1978b) was not GLP compliant, and some guideline or reporting deviations were observed, studies are overall considered to be sufficiently reliable and suitable for use in hazard classification (apart perhaps from the *Chironomus* sediment study). The endpoints based on nominal concentrations,

where measured values deviated from 80-120% of nominals, should however be used with some caution (discussed below).

This prolonged fish toxicity test guideline has subsequently been deleted by OECD but the study is included as supporting information.

11.6.1 Chronic toxicity to fish

Two studies have been submitted on the chronic toxicity of esfenvalerate to fish. One of these was carried out with rainbow trout (*Oncorhynchus mykiss*, tested as *Salmo gairdneri*) and was undertaken following the now deleted OECD 204 prolonged juvenile fish growth test guideline (1984) and to GLP. Measured concentrations in test media covering the whole test duration were not reported, however its resulting NOEC value of 0.001 µg/L (based on nominal concentrations) was the lowest amongst the two studies. The other true chronic study was a full fish life cycle test carried out with fathead minnow (*Pimephales promelas*) following the US EPA guideline (1971): 'Recommended bioassay procedure for fathead minnows (*Pimephales promelas*, Rafinesque) chronic tests'. It was not performed according to GLP but is otherwise considered reliable; this study gave a mean measured NOEC for the minnow of 0.09 µg/L. Whilst the prolonged OECD 204 guideline is no longer considered applicable as a chronic test, since its endpoint for trout is the lowest and most precautionary fish NOEC available and trout were more acutely sensitive than fathead minnow, it will be considered here. Further details of these studies can be found in Annex 1 Section 4.4.3 of this CLH report.

11.6.2 Chronic toxicity to aquatic invertebrates

Two studies have been submitted on the chronic toxicity of esfenvalerate to *Daphnia magna*. One of these was undertaken following the US EPA 72-4 (1985) guideline, and the other one followed OECD 202 Part II (1985). Both studies were conducted according to GLP standards and the lowest NOEC value of 0.0018 µg/L resulted from the study by Handley *et al* (1991). This endpoint was based on nominal concentrations and there was not clear confirmation of measured concentrations in actual test media. However, as it represents the lowest chronic NOEC value amongst all aquatic invertebrate tests, it will be considered for classification purposes. Further details of this study can be found in Annex 1 Section 4.4.4 of this CLH report.

11.6.3 Chronic toxicity to algae or other aquatic plants

A study has been submitted on the toxicity of esfenvalerate to green algae (*Scenedesmus subspicatus*), see 11.5.3 above. This study was undertaken following the OECD 201 (1984) guideline, and the resulting NOEC value (growth rate and biomass) was 1.0 µg/L based on nominal concentrations. Mean measured concentrations exceeded 120% of nominal throughout the test duration and so the nominal endpoint is potentially unreliable, however because of this it is likely to be sufficiently precautionary. Further details of this study can be found in Annex 1 Section 4.3.3 of this CLH report.

11.6.4 Chronic toxicity to other aquatic organisms

A study was submitted on the chronic toxicity of esfenvalerate to non-biting midge (*Chironomus riparius)*. This study was performed according to GLP standards and following the BBA "Effects of plant protection products on the development of sediment-dwelling larvae of *Chironomus riparius* in a water-sediment system" (1995) guideline. This study was compared to the OECD 219 by the RMS during the RAR review and was deemed acceptable. The resulting NOEC value was determined to be 0.160 µg/L (emergence), based on initial nominal concentrations in the water phase. Recoveries in the overlying water were 61.9% - 95% of the nominal concentrations at test initiation. At test termination these declined to between 3% - 6% of nominals. As mean measured endpoints in the water phase have not been determined, these results are of uncertain relevance for hazard classification. Further details of this study can be found in Annex 1 Section 4.5 of this CLH report.

Two valid GLP studies on the endocrine disrupting (ED) potential of technical esfenvalerate have been reported in the consolidated 2014 RAR for the substance under Reg.n 1107/2009. Where such studies also demonstrate 'standard' long-term/chronic classification endpoints (e.g. related to growth and reproduction)

they may be 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). Full details are only available from the RAR, however along with gonad size/histopathology, secondary sexual characteristics and plasma vitellogenin (VTG) there were also assessments of survival, wet weight and reproductive endpoints (no. spawns, no. eggs produced, no. eggs per spawn and percent fertilization). The mean measured concentrations of esfenvalerate during the 21-day exposure period were 0.0272, 0.0514 and 0.231 µg a.s./L and no statistically significant adverse effects compared to the control were reported for any of the biological endpoints. The overall measured NOEC was therefore 0.231 µg a.s./L, the highest concentration tested. As this is greater than the lowest prolonged and chronic fish NOECs of 0.001 and 0.09 µg/L respectively (see 11.6.1 above) this does not influence the proposed chronic hazard classification.

The second ED study reported in the RAR is a 21-day amphibian metamorphosis assay (to U.S. EPA and OPPTS guideline 890.1100 (2009)) by D. J. Fort (2012). The test design entailed exposing tadpoles of *Xenopus laevis* under flow-through conditions to three concentrations of technical esfenvalerate, which, based on time-weighted mean measured concentrations, were 0.0135, 0.0180 and 0.0397 µg a.s./L. The primary endpoints were hind limb length, body length (snout to vent [SVL]), developmental stage, wet weight, thyroid histology and daily mortality. The RMS reported some analytical inconsistency at the lowest two test concentrations, however, there were no reported endocrine or other survival or growth effects at any of the higher reliable test concentrations and so, based on time-weighted mean measured concentrations, the overall NOEC for esfenvalerate was 0.0397 µg a.s./L (the highest concentration tested). This again is not considered to affect the chronic hazard classification proposal.

11.7 Comparison with the CLP criteria

Esfenvalerate is hydrolytically stable under acidic conditions, but undergoes hydrolysis under neutral and alkaline conditions across all temperature ranges (>10 % degradation after 5 days). It undergoes rapid aqueous photolytic degradation to primary degradates, with low volatilisation occurring. It is not readily biodegradable (0% CO_2 evolution at 28 d). 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. Consequently, esfenvalerate is considered 'not rapidly degradable' for the purpose of classification and labelling.

The identified main degradants are less acutely toxic than the parent substance (by at least an order of magnitude) and therefore they are not considered further in relation to the classification of esfenvalerate.

The Log K_{OW} of esfenvalerate is 6.24 and above the CLP log K_{OW} trigger value of ≥4. The representative experimental whole fish BCF of 3110 of esfenvalerate is also well above the trigger value of ≥500. Esfenvalerate is therefore considered potentially bioaccumulative according to the CLP criteria.

11.7.1 Acute aquatic hazard

Sufficiently reliable acute/short-term aquatic toxicity data on esfenvalerate are available for fish, *Daphnia magna* and green algae. Fish and invertebrates were the most acutely sensitive trophic groups. Four reliable although variable 48-hour EC_{50} endpoints are available on the acute toxicity of technical esfenvalerate to *D. magna*, these were 27, 3.5, 0.9 and ≈0.045 µg/L. Since there are four endpoints for the same species, a geomean acute toxicity value for aquatic invertebrates of 1.4 µg/L has been determined. Fish are therefore considered more acutely sensitive overall with a 96-hour LC_{50} value for rainbow trout of 0.10 µg/L (equivalent to 0.0001 mg/L). On the basis of this acute fish endpoint being in the range 0.00001 mg/L $< L/EC_{50} \leq$0.0001 mg/L, esfenvalerate should be classified for acute environmental (aquatic) effects under CLP as:

Acute Category 1 with an Acute M-factor of 10000

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Sufficiently reliable chronic/long-term toxicity data are also available for fish, an amphibian, aquatic invertebrates (*Daphnia magna* and *Chironomus riparius*) and algae. The most sensitive species overall was rainbow trout with a NOEC of 0.001 µg/L (equivalent to 0.000001 mg/L). However, as this result was taken from a prolonged juvenile fish growth test (which is no longer considered truly chronic) the classification could be based on the next lowest NOEC of 0.0018 µg/L (0.0000018 mg/L) for *D. magna* (the RAC's opinion on this choice of 'chronic' endpoint is requested). On the basis of the *Daphnia* endpoint being in the range 0.000001 mg/L <NOEC ≤0.00001 mg/L, and it being 'not rapidly degradable' as well as potentially bioaccumulative, esfenvalerate would be classified under CLP as:

Chronic Category 1 with a Chronic M-factor of 10000

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Aquatic Acute Category 1; H400: Very toxic to aquatic life

Acute M-factor = 10000

Aquatic Chronic Category 1; H410: Very toxic to aquatic life with long lasting effects

Chronic M-factor = 10000

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)
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 $0.000001 < L(E)C_{50} \leq 0.00001$ 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.

<u>Aquatic Acute</u>

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 $0.00001 < L(E)C50 \leq 0.0001$ 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 $0.000001 < NOEC \leq 0.00001$ mg/L range, the **chronic M-factor is 10000.**

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

Not considered.

13 ADDITIONAL LABELLING

Not required.

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15 ANNEXES & APPENDICES

Appendix 1: Evaluation of the toxicological significance of testicular interstitial (Leydig) cell tumours observed in a rat two-year chronic/carcinogenicity dietary study

Appendix 2: Parent and environmental degradant information

Appendix 3: Aquatic toxicity data for esfenvalerate degradants

Annex 1: Robust study summaries (separate document)

Annex 2: Confidential references (separate document – CONFIDENTIAL)

Appendix 1: Evaluation of the toxicological significance of testicular interstitial (Leydig) cell tumours observed in a rat two-year chronic/carcinogenicity dietary study

LLT-0256

Document Title

Esfenvalerate;

Evaluation of the toxicological significance of testicular interstitial (Leydig) cell tumours observed in a rat two-year chronic/carcinogenicity dietary study

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19th May 2015

Title: Esfenvalerate; Evaluation of the toxicological significance of testicular interstitial (Leydig) cell tumours observed in a rat two-year chronic/carcinogenicity dietary study

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1. Summary

Esfenvalerate is a synthetic pyrethroid which is used as an insecticide. Classification of esfenvalerate for carcinogenicity was proposed as "Carcinogenicity Category 2 (Carc. Cat.2) in the EFSA Conclusion (EFSA Journal, 2014). This document sets forth the notifier's opinion on the proposed classification based on evaluation of the toxicological significance of testicular interstitial (Leydig) cell tumours observed in a Wistar rat two-year chronic/carcinogenicity dietary study with esfenvalerate.

The tumourigenic potential of esfenvalerate was investigated in standard bioassays using male and female rats and mice in compliance with Good Laboratory Practice, the test guidelines and protocol designated by authorities. While the conclusion that esfenvalerate has no tumourigenic potential in mice was accepted, ***testicular Leydig cell tumours observed in the rat two-year study with esfenvalerate were considered to be treatment-related in the EFSA conclusion***. The testicular Leydig cell tumours were observed in 2, 1, 0, 4, and 4 animals at 0 (control), 15, 50, 150 and 400 ppm, respectively. The incidence in the control group was 4% and in the 150 and 400 ppm groups the incidences were 27% and 8 %, respectively. This resulted in a statistically significant difference at 150 ppm but not at 400 ppm because the number of animals examined at 150 ppm was limited (only 15 animals of interim death or sacrifice). Recently, an additional histopathological examination at 15, 50 and 150 ppm was conducted on testis tissues collected in remaining animals to complete these investigations for all main study animals. The additional examination did not reveal new pre-neoplastic or neoplastic lesions in the testis. Consequently, Fisher's exact test revealed no statistical significance ($p>0.05$) at 150 ppm as well as 400 ppm. The corresponding incidences were 4%, 2%, 0%, 8% and 8% at 0, 15, 50, 150 and 400 ppm, respectively. These incidences showed no clear dose response and were within the laboratory's historical control range for the Wistar strain (0-10%).

A possible carcinogenic potential of esfenvalerate is comprehensively discussed in this document. One tumour type (benign) in one sex (male) of one species (the rat but not mouse) occurred in one study with esfenvalerate. Esfenvalerate is non-genotoxic. Four cases of benign Leydig cell tumours occurred in male rats exposed to each of 150 and 400 ppm esfenvalerate, respectively. However, these findings are considered not to represent a carcinogenic potential based on the following evidence (1) no statistically significant differences between control and treatment groups; (2) incidences are within the historical control range of the laboratory and the published range for Wistar rats; (3) no significantly increased pre-neoplastic changes (Leydig cell hyperplasia); (4) no increased testis weight in the acute and long-term studies, including the rat 2-year study; (5) no accumulation or persistence of esfenvalerate and its metabolites in the testes based on the results of metabolism studies; and (6) the known modes of action via endocrine imbalance are unlikely, as demonstrated by no interaction with the Hypothalamic-Pituitary-Gonadal (HPG) axis and no reproductive abnormality.

In conclusion, based on strong weight of evidence considerations, the testicular Leydig cell tumours observed in the rat 2-year study are considered not to be treatment-related. Therefore, it is concluded that, in light of current criteria and available data, esfenvalerate should remain "Not classified for carcinogenicity" and does not warrant classification as a "Category 2 carcinogen".

2. Introduction

Esfenvalerate is a synthetic pyrethroid which is used as an insecticide. The tumourigenic potential of esfenvalerate has been studied in male and female rats and mice in compliance with Good Laboratory Practice, the test guidelines and protocols designated by the European Community (EC), Organisation for Economic Co-operation and Development (OECD), US. Environmental Protection Agency (US.EPA), and Ministry of Agriculture, Forestry and Fisheries of Japan (Japan MAFF).

In reference to the testicular Leydig cell tumours observed in a rat 2-year chronic/carcinogenicity dietary study, esfenvalerate was proposed to be classified as "Carcinogenicity Category 2 (Carc. Cat.2)" in the EFSA Conclusion (EFSA Journal, 2014).

This document sets forth the notifier's opinion concerning the above proposed classification based on evaluation of the toxicological significance of testicular Leydig cell tumours observed in the rat 2-year study with esfenvalerate. It considers all the pertinent toxicology reports and results relating to testicular adenomas, historical control data of rat 2-year studies from the laboratory where the study was conducted (previously Harlan Laboratories, Switzerland), together with published information.

Based on these considerations, the slightly increased incidence of testicular Leydig cell tumours observed in the rat 2-year study with esfenvalerate is considered not to be treatment related and is not toxicologically significant. Therefore, it is concluded that esfenvalerate shows no carcinogenic effect and thus classification as a Category 2 carcinogen is not warranted.

3. Data analysis of carcinogenicity study in the rat[redacted], 2011)

3.1 Study design

Male and female Wistar rats (50 animals/dose/sex) were fed 0 (control), 15, 50, 150, or 400 ppm esfenvalerate (purity, 87.3%) in the diet for 104 weeks (average chemical intakes: 0.7, 2.3, 6.9, and 18.5 mg/kg/day for males; 0.8, 2.7, 8.0 and 21.5 mg/kg/day for females, respectively). In addition, an interim necropsy was conducted on additional animals in each dose level (20 animals/dose/sex) at the end of week 52

of the study. Based on the results of two 13-week feeding studies in rats (Kelly, 1985; Larson, 1987) and a 4-week feeding study in rats (Sommer, 2008), the dose levels described above were selected. In the 13-week feeding studies, rats dosed at 500 ppm exhibited mortality, decreased body weights and clinical signs such as jerky leg movement. These effects were much less pronounced in rats dosed at 300 ppm and no mortality was observed. In the 4-week feeding study, mortality was observed in the 700 and 1000 ppm groups. Decreases in body weights and clinical signs were exhibited in the groups dosed at or higher than 500 ppm. Therefore, 400 ppm was chosen as the highest dose level to elicit evidence of toxicity over the course of the carcinogenicity study without altering the animals' normal lifespan. Lower dose levels of 150 ppm, 50 ppm and 15 ppm were selected according to a common ratio of approximately 3 to examine the dose response and establish a no-observed-adverse-effect-level (NOAEL).

3.2 Suitability of dose levels tested

Under the conditions of this study, there were no treatment-related effects on mortality, clinical signs or behaviour with esfenvalerate for up to 104 weeks. Overall mean food consumption in treated males and females was not affected by treatment with esfenvalerate. However, clear treatment-related effects on body weight were observed in males at 400 ppm, and similar but slight effects were also observed in females at 400 ppm without statistical significance. Treatment-related effects on body weight gain were also observed in males and females at 400 ppm. Overall mean body weight gains in males and females were suppressed by approximately 10% by treatment with esfenvalerate. Taken together with exhibited mortality at 500 ppm in the 13-week study and clinical signs and decreased body weight at 500 ppm in the 4-week study, the body weight changes in this study indicated that the dose level of 400 ppm was a maximum tolerated dose (MTD) and was suitable to determine the carcinogenic potential of esfenvalerate.

3.3 Testicular Leydig cell tumours observed

At 15, 50 and 150 ppm, the original histopathological examination had been conducted on testes from animals that were found dead or killed in a moribund condition during the treatment period and from animals with gross lesions in testes at terminal necropsy after the 104-week treatment (Sommer, 2011). Recently, an additional histopathological examination was conducted on testis tissues collected in remaining animals at these dose levels to complete these investigations for all main study animals (Amended report for Sommer (2011); Kaiser, 2015). The additional examination did not reveal new pre-neoplastic or neoplastic lesions in the testis. As shown in Tables 1 and 2, testicular Leydig cell tumours were observed in 2, 1, 0, 4, and 4 animals at 0 (control), 15, 50, 150 and 400 ppm, respectively. All of them

excluding one animal (Animal No. 248 of 150 ppm, sacrificed at Day 668) were observed at terminal sacrifice after the two-year treatment. All these tumours were benign (adenoma), not malignant, and were observed unilaterally. Fisher's exact test revealed no statistical significance ($p>0.05$). No dose-dependency was also evident (no statistical significance) by Peto analysis ($p>0.05$).

Table 1. Incidence of testicular Leydig cell tumour and hyperplasia in the rat 2-year study with esfenvalerate

Finding	Level (ppm)	0	15	50	150	400
	Nos. of rats examined:	50	50	50	50	50
Benign Leydig cell tumour	Numbers of rats with finding	2	1	0	4	4
	Incidence (%)	(4)	(2)	(0)	(8)	(8)
Malignant Leydig cell tumour	Numbers of rats with finding	0	0	0	0	0
	Incidence (%)	(0)	(0)	(0)	(0)	(0)
Leydig cell hyperplasia	Numbers of rats with finding	2	1	0	1	0
	Incidence (%)	(4)	(2)	(0)	(2)	(0)
Animals bearing Leydig cell tumour or hyperplasia	Numbers of rats with finding	3	1	0	4	4
	Incidence (%)	(6)	(2)	(0)	(8)	(8)

At 15, 50 and 150 ppm, original histopathological examination had been conducted on testes from animals that were found dead or killed in a moribund condition during the treatment period and from animals with gross lesions in testes at terminal necropsy after 104-week treatment. An additional histopathological examination at these doses was conducted on testis tissues collected in remaining animals to complete these investigations for all main study animals.

Fisher's exact test; Not significant ($p>0.05$),

Peto test; Not significant ($p>0.05$)

Table 2. Individual data of animals bearing testicular Leydig cell hyperplasia or tumour in the rat 2-year study with esfenvalerate

Control		15 ppm		50 ppm		150 ppm		400 ppm	
Animal No	Findings	Animal No	Findings	Animal No	Findings	Animal No	Findings	Animal No	Findings
37	Ad	102	Hy, Ad	-	-	236	Ad	303	Ad
43	Hy, Ad	-	-	-	-	243	Hy, Ad	307	Ad
46	Hy	-	-	-	-	248	Ad	323	Ad
-	-	-	-	-	-	250	Ad	327	Ad

Hy: Hyperplasia, Ad: Adenoma

When the tumourigenicity of esfenvalerate is evaluated, comparison with historical background data apart from concurrent control is also useful because tumour incidences can have considerable biological variation. Historical control data in Wistar rats at Harlan Laboratories are shown in Table 3 and Figure 1. The relevant guidelines address the topic of genetic drift directly or indirectly and define a time window of 2–3 years prior to or following the study (USEPA, 2005), 5 years prior to the study (EMEA, 2002), 5 years prior to and 2–3 years following the study (OECD, 2002). However, Deschl *et al.* (2002) demonstrated that some parameters may change in a short period of time while others remain stable over prolonged periods. Furthermore, Nolte *et al.* (2011) concluded that a time window should be defined for each lesion after analysis of the dependency between incidence and year of study start. The use of a "fixed moving time window" may lead to loss of important information or the reference to inappropriate historical control data. We evaluated the correlation between Leydig cell tumour incidence and year of study start. As shown in Figure 1, there are low correlations (R^2=0.0602) on the background values for the tumours in Harlan Laboratories. Thus the present analysis revealed no dependency between year of study start and incidence of these tumours for this laboratory, indicating stability of the background incidence over time and, therefore, no need to apply a time window when defining the reliability of historical control data.

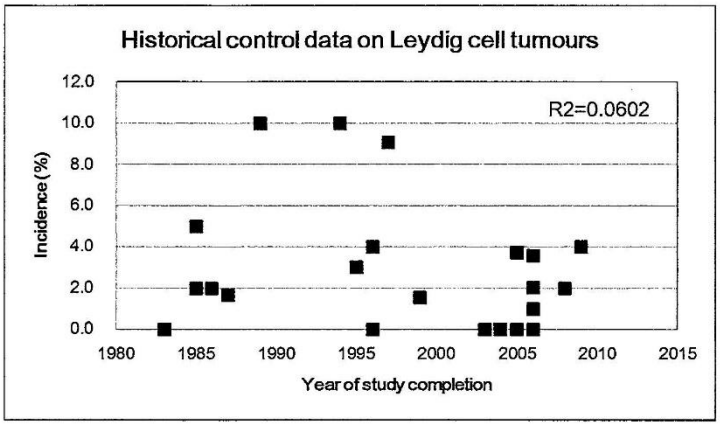
Table 3. Historical background values of testicular Leydig cell tumour in male Wistar ratsfrom 2-year feeding studies at Harlan Laboratories (Harlan 2010)

Study ID #	2	3	6	8	14	24	32	33	34	35	36	37
Completed year	1983	1985	1985	1986	1987	1989	1994	1995	1997	1996	1996	1999
Pathologist	JMA	RUD	HHW	JMA	BSC	JMA	JMA	HHW	JMA	HJC	HHW	JMA
Numbers of rats examined	99	100	50	100	60	70	50	100	99	50	60	64
Leydig cell tumor (b)	0	5	1	2	1	7	5	3	9	2	0	1
%	0.0	5.0	2.0	2.0	1.7	10.0	10.0	3.0	9.1	4.0	0.0	1.6
Leydig cell tumor (m)	0	0	0	0	0	0	0	0	0	0	0	0
%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Leydig cell hyperplasia	1	1	0	3	5	3	11	0	4	0	0	3
%	1.0	1.0	0.0	3.0	8.3	4.3	22.0	0.0	4.0	0.0	0.0	4.7

Study ID #	39	40	41	42	43	44	45	48	50	51	53
Completed year	2003	2004	2005	2004	2006	2006	2006	2005	2006	2008	2009
Pathologist	WEK	JMA	WEK	WEK	WEK	WEK	KHE	HJC	WEK	WEK	WEK
Numbers of rats examined	70	50	50	50	112	100	99	107	50	50	100
Leydig cell tumor (b)	0	0	0	0	4	1	2	4	0	1	4
%	0.0	0.0	0.0	0.0	3.6	1.0	2.0	3.7	0.0	2.0	4.0
Leydig cell tumor (m)	1	0	0	0	0	0	0	0	0	0	0
%	1.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Leydig cell hyperplasia	0	3	0	1	2	1	1	0	0	0	1
%	0.0	6.0	0.0	2.0	1.8	1.0	1.0	0.0	0.0	0.0	1.0

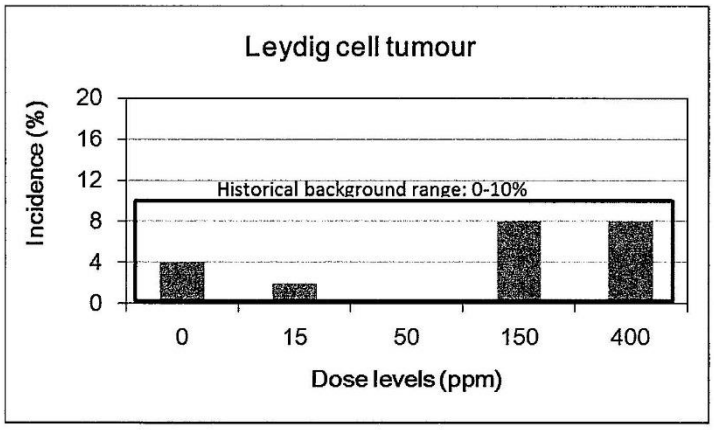
(b) benign, (m) malignant

Figure 1. Historical background values of testicular benign Leydig cell tumours in male Wistar rats from 2-year feeding studies at Harlan Laboratories (Harlan 2010)



Total number of studies: 23
Total number of animals examined: 1740
Number of animals bearing benign Leydig cell tumour: 52
Total incidence: 3.0 %
Mean value ±SD of incidence: 2.8 ±3.1 %
Range: minimum 0.0%, maximum 10.0%

Figure 2. Incidence of testicular Leydig cell tumours in the rat 2-year study with esfenvalerate and comparison with historical control data



The incidence of testicular Leydig cell tumours at 150 and 400 ppm (4 of 50 animals per group; 8 %) was within the historical range of this laboratory (0-10.0%). Nolte *et al.* demonstrated that the incidence was highly variable in Wistar rats with a minimum of 0%, a maximum of 60% and a mean of 13.7% (Nolte *et al.*, 2011; see

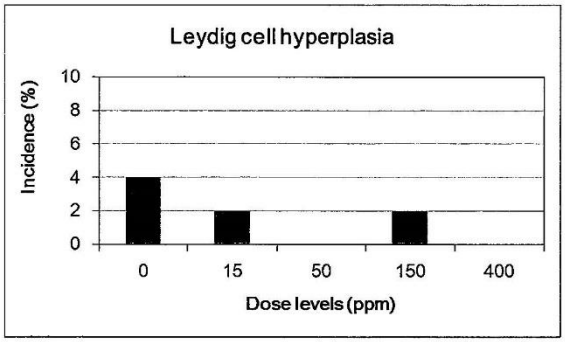
below Table 4). Haseman at NTP suggested using $p<0.01$ rather than $p<0.05$ for significance to avoid false positives if a spontaneous incidence is more than 1% (Haseman, 1983). In the rat 2-year study with esfenvalerate, Fisher's exact test and Peto analysis revealed no statistical significance ($p>0.05$) for the incidence of testicular Leydig cell tumours. These findings strongly support the absence of a relationship between esfenvalerate treatment and appearance of testicular Leydig cell tumours.

Table 4. Strain- and breeder- dependent differences in the incidence of testicular Leydig cell adenoma (Nolte *et al.*, 2011)

Strain	Breeder	*N* studies	*N* animals	Incidence [%]		
				Mean	Min.	Max.
Wistar	A	19	1079	39.9	18	60
	B	12	800	2.8	0	6
	D1	11	608	5.8	0	29
	G	16	770	12.5	4	22
	I	30	1609	6.8	1.1	22
SD	D1	10	518	5.4	0	12
	D2	5	279	5	1.7	10
	D3	8	440	2.3	1.8	3.6
	H	6	398	6.3	2.0	8.6
F344	D6	2	100	83	76	90

Testicular Leydig cell hyperplasia is often observed as a pre-neoplastic finding related to testicular Leydig cell tumours. In the 2-year rat study with esfenvalerate, while testicular Leydig cell hyperplasia was also observed in 2, 1, 0, 1, and 0 animals at 0 (control), 15, 50, 150 and 400 ppm, respectively, there were no statistically significant changes (Table 2, Figure 3). Thus there is clearly no treatment related effect on the incidence of testicular Leydig cell hyperplasia.

Figure 3. Incidence of testicular Leydig cell hyperplasia in the rat 2-year study with esfenvalerate



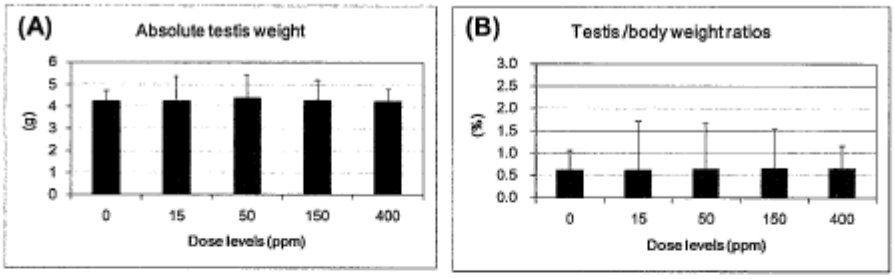
Fisher exact test; No significant difference from control ($p>0.05$)

In the case of testicular Leydig cell tumours, many pathologists agree that the distinction between hyperplasia and adenoma is often based primarily on the size of the lesion since cytologic features are usually similar between hyperplasia and adenoma (Cook *et al.*, 1999). Because the distinction is less precise, it is important to emphasise that this is more readily understandable when it is appreciated that such interstitial lesions represent a morphological continuum that begins with hyperplasia that can progress to the formation of an adenoma. When testicular proliferative lesions are assessed as combined incidence of testicular Leydig cell hyperplasia and/or tumour, the lesions are observed in 3, 1, 0, 4 and 4 animals in control, 15, 50, 150 and 400 ppm esfenvalerate groups, respectively (see Table 2). The difference between the control and the 2 higher dose groups is only one animal, demonstrating that there is no convincing evidence of a treatment related effect on the incidence of proliferative lesions of testicular Leydig cells.

3.4 Testicular weight

Absolute and relative (organ/body weight ratio) weights of testes in the rat 2-year study with esfenvalerate are shown in Figure 4. When proliferation of Leydig cells is enhanced, testicular weight may be increased. However, there were no significant differences between control and treated groups.

Figure 4. Testicular weight [absolute (A), relative (testis/body weight ratios) (B)] of animals after 104-week treatment in the rat 2-year study with esfenvalerate



The Dunnett-test; No significant difference from control (p>0.05)

4. Weight of evidence for no treatment-related increase of testicular Leydig cell tumour

4.1 Rat carcinogenicity study [redacted] 2011; [redacted] 2015)

Benign Leydig cell tumours and Leydig cell hyperplasia, but not malignant Leydig cell tumours, were observed in some animals of the rat 2-year study with esfenvalerate. As discussed above, however, there is no statistical significance between control

and treatment groups. The incidence of this tumour observed at 150 and 400 ppm in the rat 2-year study with esfenvalerate was within the historical control range of the laboratory (Harlan, 2010) and the published range for Wistar rats (Notle et al., 2011). The benign adenomas occurred at a late stage in the studies and the treatment with esfenvalerate did not cause a reduction in the latency of onset. While increased testis weight is expected if a chemical induces testicular tumours, there was no increased tendency in this study. Furthermore, there was no treatment related effect on the incidence of Leydig cell hyperplasia. These findings demonstrate that there was no evidence of treatment-related tumourigenic effects on testes in the rat 2-year study with esfenvalerate.

4.2 Mouse carcinogenicity study [redacted] 1997)

There were no testicular lesions or other indications of carcinogenesis.

4.3 Genotoxicity (Kogiso, 1985a,b,c,d; Kogiso, 1986)

Esfenvalerate was not genotoxic in a battery of *in vitro* and *in vivo* assays: reverse mutation test in a bacterial system, chromosomal aberration test in Chinese Hamster ovary cells (CHO-K1), gene mutation test in Chinese Hamster V79 cells, unscheduled DNA synthesis (UDS) assay in HeLa cells, and micronucleus test in mice.

4.4 Metabolism in rats [redacted] 1985; [redacted] 1998)

The absorption, metabolism, distribution, and excretion of esfenvalerate were investigated in rats and mice. Excretion was very rapid with 78 – 95% of the administered label being excreted within one day after dosing and virtually complete elimination occurred by days 6-7. Tissue residues were generally very low and oral absorption was considered to be 64%. There were no major sex differences in metabolism.

Therefore, it is considered that accumulation and persistence of esfenvalerate or its metabolites in testes that could induce testicular dysfunction is unlikely to occur in the rat 2-year study.

4.5 No evidence of known modes of action for testicular Leydig cell tumourigenicity

There are a number of potential mechanisms whereby chemicals might induce testicular Leydig cell tumours, primarily through a disruption in the Hypothalamic-

Pituitary-Testis (HPT) axis (Cook *et al.*, 1999; Foster 2007). In these mechanisms, most chemicals induce an elevation in circulating luteinizing hormone (LH) levels. Increased LH has long been known to be able to produce testicular Leydig cell hyperplasia and tumours if the elevations are over a sustained period (Cook *et al.*, 1999; Foster 2007). Therefore, for a better understanding of the tumourigenic potential of esfenvalerate on rat testes, assessment for effects of esfenvalerate on the HPT axis should be very useful. Plausible mechanisms for the chemical induction of testicular Leydig cell tumours are typified by agonists of estrogen, gonadotropin releasing hormone (GnRH), and dopamine receptors, androgen receptor antagonists, and inhibitors of 5α-reductase, testosterone biosynthesis, and aromatase. Most of these ultimately involve elevation in serum LH and/or testicular Leydig cell responsiveness to LH as proximate mediators (Cook *et al.*, 1999).

4.5.1 General and reproductive toxicity studies

In general toxicity studies with esfenvalerate, the effects on organ weights (pituitary and gonad including testis) and histology (hypothalamus, pituitary and gonad including testis) were investigated. In reproductive toxicity studies, the effects on mating indices, fertility indices and reproductive organs were investigated. Based on the results of the above-mentioned studies, a potential to interact with the endocrine system, including testis, was not detected. Therefore, there were no obvious correlating factors in the toxicology or metabolism reported for esfenvalerate that could be considered to predispose to the testicular findings in rats.

4.5.2 Endocrine disruption studies (Willoughby, 2012; [redacted] 2012; Snajdr, 2011a,b; [redacted] 2012a,b; [redacted] 2001; [redacted] 2012; [redacted] 2012)

A comprehensive suite of studies with esfenvalerate was conducted to investigate endocrine disruption potential as part of US EPA's Endocrine Disruptor Screening Program (EDSP). These studies were conducted in accordance with the Series 890-EDSP Test Guidelines and/or OECD Test guidelines for male/female pubertal, Hershberger, steroidogenesis, androgen/estrogen receptor binding, uterotrophic, aromatase, estrogen receptor transcriptional activation, fish short-term reproduction, and amphibian metamorphosis assessment. Based on the results of these studies, esfenvalerate did not exhibit any evidence of endocrine mediated effects in a suite of *in vitro* and *in vivo* studies. Some of studies can sensitively assess plausible mechanisms for the chemical induction of testicular Leydig cell tumours typified by agonists of estrogen, gonadotropin releasing hormone (GnRH), and dopamine receptors, androgen receptor antagonists, and inhibitors of 5α-reductase, testosterone biosynthesis, and aromatase. A male pubertal assay is capable of detecting (anti-)androgenic chemicals or chemicals which alter pubertal development via changes in gonadotropins, prolactin, hypothalamic /pituitary /testicular function. In particular, the functional effects on testes were not detected in parameters of serum testosterone level, preputial separation, organ weight and histology. Consequently, it

was considered that esfenvalerate does not have the potential to interact with the endocrine system.

5. Implications for classification

Based on the ECHA guidance documents, the implications of the findings from the various bioassay studies on the classification of esfenvalerate are summarized in Table 5. In the absence of a significant increase in benign Leydig cell tumours and any malignant Leydig cell tumours in the rat carcinogenicity study it can be concluded that, based on current criteria and the available evidence, it is clear that esfenvalerate should remain "Not classified for carcinogenicity".

Table 5. Summary of the findings from the bioassay studies on the classification of esfenvalerate

Criteria for classification (ECHA, 2013)	**Findings from the bioassay studies with esfenvalerate**
Tumour type and background incidence	Testicular Leydig cell adenoma The incidence was within the historical control range
Multi-site responses	No (testis only, unilateral)
Progression of lesions to malignancy	No (benign only)
Reduced tumour latency	No (the tumours occurred at a later stage of the study; indeed, all of the findings were observed at terminal necropsy after two-year treatment except one animal; interim sacrificed animal at Day 668 in 150 ppm group)
Whether responses in single or both sexes	Single sex only (males)
Whether response single or several species	Single species only (rat)
Structural similarity to a substance(s) for which there is good evidence of carcinogenicity	The Agency for Toxic Substances and Disease Registry (ATSDR) provided an excellent review entitled "Toxicological Profile for Pyrethrins and Pyrethroids." According to this review, no reports were located regarding cancer in humans or animals following inhalation or dermal exposure to pyrethrins or pyrethroids (ATSDR, 2003). However, in the case of oral exposure to these chemicals, while no reports were located regarding cancer in humans, pyrethrins and some pyrethroids have been

Criteria for classification (ECHA, 2013)	**Findings from the bioassay studies with esfenvalerate**
	shown to cause tumours in rodent models. Most of the pyrethroids are not carcinogenic. However, some pyrethroids and pyrethrins increased the production of tumours in rodents. These data indicate that tumour induction does not appear to reflect a common carcinogenic endpoint for this particular subset of compounds (Tsuji et al., 2011). Fenvalerate (esfenvalerate is one of the 4 isomers of fenvalerate) increase in Leydig cell tumours in SLC:Wistar rats but the SCP agreed that since this was only seen in a strain with a high susceptibility to Leydig cell tumours that the effect was not relevant for man (SCP/ESFEN/002-Final). This is analogous to the common situation with F344 rats.
Routes of exposure	Dietary is relevant to man
Comparison of absorption, distribution, metabolism and excretion between test animals and humans	Esfenvalerate was eliminated primarily via NADPH-dependent oxidative metabolism in both rat and human liver microsomes. The intrinsic hepatic clearance of esfenvalerate was estimated to be 3-fold greater in rodents than in humans on a per kilogram body weight basis (Godin et al., 2006).
Possibility of a confounding effect of excessive toxicity at test doses	No
Mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity.	Not genotoxic. No evidence of cytotoxicity or cell proliferation. Mode of action not elucidated as tumours are considered not to be treatment related

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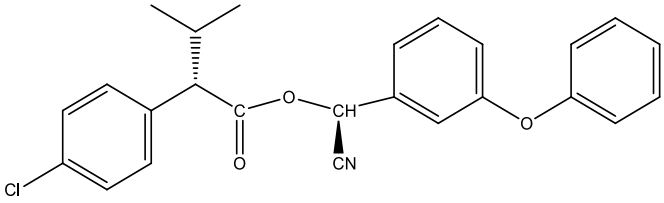
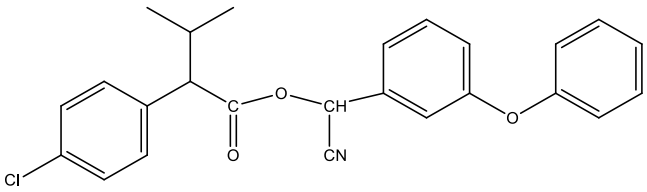
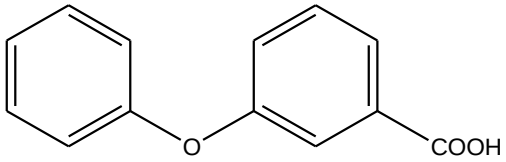
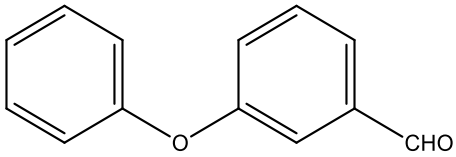
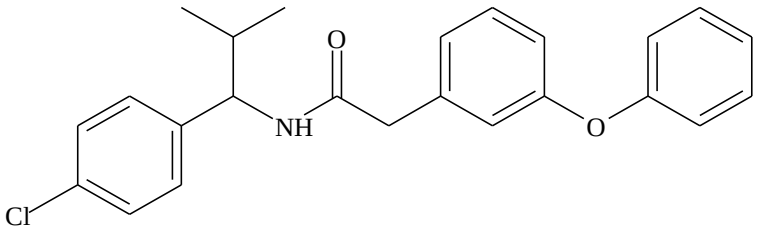
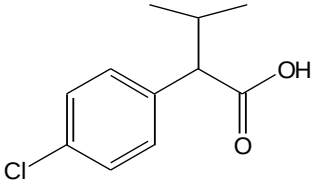
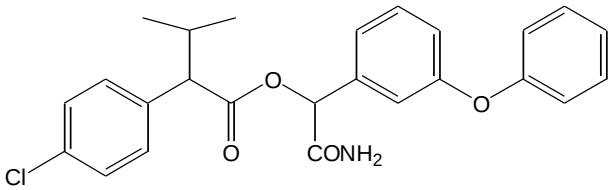
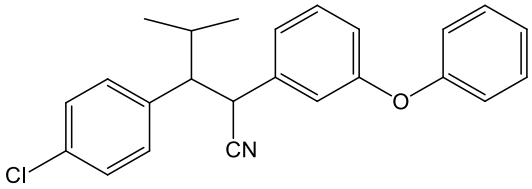
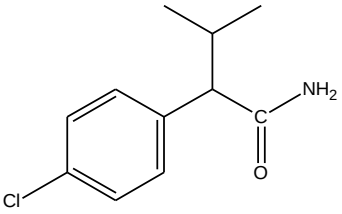
End of Report

Appendix 2 – Parent and environmental degradant information

Substance	Report name, Structure IUPAC name	Molecular formula molecular weight
Esfenvalerate (parent substance)	 *(S)*- α-cyano-3-phenoxybenzyl *(S)*-2-(4-chlorophenyl)-3-methylbutyrate Fenvalerate  *(RS)*- α-cyano-3-phenoxybenzyl *(RS)*-2-(4-chlorophenyl)-3-methylbutyrate	$C_{25}H_{22}ClNO_3$ Mw = 419.91
PBacid	 3-phenoxybenzoic acid	$C_{13}H_{10}O_3$ Mw= 214.2
PBald	 3-phenoxybenzaldehyde	$C_{13}H_{10}O_2$ Mw= 198.2
PA-Fen	 *N*-[1-(4-chlorophenyl)-2-methylpropyl]-3-phenoxyphenylacetamide	$C_{24}H_{24}O_2NCl$ Mw= 393.9
CPIA	 2-(4-chlorophenyl)isovaleric acid	$C_{11}H_{13}O_2Cl$ Mw = 212.7

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON ESFENVALERATE (ISO); (*S*)-α-CYANO-3-PHENOXYBENZYL-(*S*)-2-(4-CHLOROPHENYL)-3-METHYLBUTYRATE

Substance	Report name, Structure IUPAC name	Molecular formula molecular weight
$CONH_2$-Fen	 2-amino-2-oxo-1-(3-phenoxyphenyl)ethyl 2-(4-chlorophenyl)-3-methylbutanoate	$C_{25}H_{24}O_4NCl$ Mw = 437.9
Dec-Fen	 3-(4-chlorophenyl)-4-methyl-2-(3-phenoxyphenyl)pentanenitrile	$C_{24}H_{22}ONCl$ Mw = 375.9
CPIA-carboxamide	 2-(4-chlorophenyl)-3-methylbutanamide	$C_{11}H_{14}ONCl$ Mw= 211.7

Appendix 3 – Aquatic toxicity data for esfenvalerate degradants.

Table 1: Summary of relevant information on aquatic toxicity for esfenvalerate degradants

Method/GLP	Species	Exposure/ endpoint	Results (µg/L)	Remarks1	Reference
3-Phenoxybenzoic acid					
Acute toxicity to fish; OECD 203; GLP	Rainbow Trout (*Oncorhynchus mykiss*)	Static/mortality	96 h LC_{50} – 14,300	mm	Anonymous (2005); LLW-0114; RAR B.9.2.1 (v)
Acute toxicity; OECD 202; GLP	*Daphnia magna*	Static/immobilisation	48 h EC_{50} – 35,400	n – supported by analytical verification	van der Kolk, J. (2005b); LLW-0115; RAR B.9.2.1 (xv)
Freshwater algal growth inhibition; OECD 201; GLP	*Pseudokirchneriella subcapitata*	Static/cell multiplication inhibition	72 h E_bC_{50} - 33,790; 72 h E_rC_{50} - 51,920	mm	van der Kolk, J. (2005d); LLW-0117; RAR B.9.2.1 (xxii)
(+)CPIA					
Acute toxicity to fish; guideline not specified; non-GLP	Killifish (*Oryzias latipes*)	Static/mortality	96 h LC_{50} – 74,100	n – no analytical verification; not accepted in RAR	Anonymous (1984c); F-84036; RAR B.9.2.1 (viii))
Acute toxicity; OECD 202; GLP	*Daphnia magna*	Static/immobilisation	48 h EC_{50} – 74,000	n – supported by analytical verification	van der Kolk, J. (2005c); LLW-0116; RAR B.9.2.1 (xvi)
Freshwater algal growth inhibition; OECD 201; GLP	*Pseudokirchneriella subcapitata*	Static/cell multiplication inhibition	72 h E_bC_{50} – 64,600; 72 h E_rC_{50} – >100,000	n – supported by analytical verification	van der Kolk, J. (2005e); LLW-0118; RAR B.9.2.1 (xxiii)
Dec-Fen					
Acute toxicity to fish; OECD 203; GLP	Rainbow Trout (*Oncorhynchus mykiss*)	Static/mortality	96 h LC_{50} – >990	mm	Anonymous (2009a); LLW-0129; RAR B.9.2.1 (vi)
Acute toxicity; OECD 202; GLP	*Daphnia magna*	Static/immobilisation	48 h EC_{50} – >860	mm	Matsuura, Y. (2009c); Y. LLW-0130; RAR B.9.2.1 (xvii)
Freshwater algal growth inhibition; OECD 201; GLP	*Pseudokirchneriella subcapitata*	Static/cell multiplication inhibition	72 h E_bC_{50} – >240; 72 h E_rC_{50} – >240	mm	Fujii, A. (2009a); LLW-0131; RAR B.9.2.1 (xxiv)
$CONH_2$-Fen					
Acute toxicity to fish; OECD 203; GLP	Rainbow Trout (*Oncorhynchus mykiss*)	Static/mortality	96 h LC_{50} – 110	mm	Anonymous (2009b); LLW-0133; RAR B.9.2.1 (vii)

Method/GLP	Species	Exposure/ endpoint	Results (µg/L)	Remarks1	Reference
Acute toxicity; OECD 202; GLP	*Daphnia magna*	Static/immobilisation	48 h EC_{50} – >930	mm	Matsuura, Y. (2009d); LLW-0134; RAR B.9.2.1 (xviii)
Freshwater algal growth inhibition; OECD 201; GLP	*Pseudokirchneriella subcapitata*	Static/cell multiplication inhibition	72 h E_bC_{50} – >150; 72 h E_rC_{50} – >150	mm	Fujii, A. (2009b); LLW-0135; RAR B.9.2.1 (xxv)
PA-Fen					
Acute toxicity to fish; OECD 203; GLP	Rainbow Trout (*Oncorhynchus mykiss*)	Static/mortality	96 h LC_{50} – >703	mm	Anonymous (2012d); LLW-0143; RAR B.9.2.1 (ix)
Acute toxicity; OECD 202; GLP	*Daphnia magna*	Static/immobilisation	48 h EC_{50} – >382	mm	Anderson, M., MacDougall, A. (2012a); LLW-0144; RAR B.9.2.1 (xix)
Freshwater algal growth inhibition; OECD 201; GLP	*Pseudokirchneriella subcapitata*	Static/cell multiplication inhibition	72 h E_bC_{50} – >421; 72 h E_rC_{50} – >421	mm	Anderson, M., MacDougall, A. (2012b); LLW-0145; RAR B.9.2.1 (xxvi)

[1] n – nominal concentration; mm – mean measured concentration