Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

2,3,5,6-tetrafluoro-4-(methoxymethyl)benzyl (1*R*,3*R*)-2,2-dimethyl-3-[(1*Z*)-prop-1-en-1-yl] cyclopropanecarboxylate; Epsilon-metofluthrin

EC Number: -
CAS Number: 240494-71-7

CLH-O-0000001412-86-111/F

Adopted

3 June 2016

3 June 2016

CLH-O-0000001412-86-111/F

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

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

Chemical name: **2,3,5,6-tetrafluoro-4-(methoxymethyl)benzyl (1*R*,3*R*)-2,2-dimethyl-3-[(1*Z*)-prop-1-en-1-yl] cyclopropanecarboxylate; Epsilon-metofluthrin**

EC Number: **-**

CAS Number: **240494-71-7**

The proposal was submitted by the **United Kingdom** and received by RAC on **4 June 2015.**

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

PROCESS FOR ADOPTION OF THE OPINION

United Kingdom has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **30 June 2015**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **14 August 2015**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Elodie Pasquier**

Co-Rapporteur, appointed by RAC: **Bogusław Barański**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **3 June 2016** by **a simple majority of all members present and having the right to vote**.

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

	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 state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	**No current Annex VI entry**										
Dossier submitters proposal	xxx-xxx-xx-x	2,3,5,6-tetrafluoro-4-(methoxymethyl)benzyl (1*R*,3*R*)-2,2-dimethyl-3-[(1*Z*)-prop-1-en-1-yl]cyclopropanecarboxylate; Epsilon-metofluthrin	-	240494-71-7	Acute Tox. 4 Acute Tox. 3 STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H332 H301 H373 (inhalation) H400 H410	GHS06 GHS08 GHS09 Wng	H332 H301 H373 (inhalation) H410	-	M=100 M=100	-
RAC opinion	xxx-xxx-xx-x	2,3,5,6-tetrafluoro-4-(methoxymethyl)benzyl (1*R*,3*R*)-2,2-dimethyl-3-[(1*Z*)-prop-1-en-1-yl]cyclopropanecarboxylate; Epsilon-metofluthrin	-	240494-71-7	Acute Tox. 4 Acute Tox. 3 STOT SE 1 STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H332 H301 H370 (nervous system) H373 H400 H410	GHS06 GHS08 GHS09 Dgr	H332 H301 H370 (nervous system) H373 H410		M=100 M=100	
Resulting Annex VI entry if agreed by COM	xxx-xxx-xx-x	2,3,5,6-tetrafluoro-4-(methoxymethyl)benzyl (1*R*,3*R*)-2,2-dimethyl-3-[(1*Z*)-prop-1-en-1-yl]cyclopropanecarboxylate; Epsilon-metofluthrin	-	240494-71-7	Acute Tox. 4 Acute Tox. 3 STOT SE 1 STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H332 H301 H370 (nervous system) H373 H400 H410	GHS06 GHS08 GHS09 Dgr	H332 H301 H370 (nervous system) H373 H410		M=100 M=100	

GROUNDS FOR ADOPTION OF THE OPINION

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

No classification is proposed by the Dossier Submitter (DS) for physical hazards based on the following observations:

- Epsilon-metofluthrin does not exhibit explosive properties according to OPPTS 830-6316.
- Epsilon-metofluthrin does not exhibit oxidizing properties according to OPPTS 830.6314.
- Epsilon-metofluthrin has a flashpoint > 110°C and does not meet criteria for a flammable liquid.
- Experience in handling and use indicates epsilon-metofluthrin is not pyrophoric and does not react with water to liberate flammable gases.

Comments received during public consultation

No specific comments were received.

Assessment and comparison with the classification criteria

RAC supports the proposal of DS **not to classify epsilon-metofluthrin for physical hazards**.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute toxicity: inhalation

An LC_{50} value of 1-2 mg/L was observed in the acute inhalation study. Given the relevant values in Annex I of CLP for inhalation of dusts and mist (1 < ATE ≤ 5 mg/L), classification as Acute Tox. 4; H332 is justified.

Acute toxicity: oral

Metofluthrin, when administered as the neat liquid substance via the oral route of exposure, caused a single mortality in rats (1/5 females) at the highest dose of 2000 mg/kg bw in a standard study (OECD TG 401). However, when rats and mice were administered single doses of epsilon-metofluthrin in corn oil, treatment-related mortalities were observed in rats (7/20 - 3 died plus 4 were sacrificed intercurrently) at the highest dose of 100 mg/kg bw and in mice (2/10) at the highest dose of 60 mg/kg bw. These findings suggest that the intrinsic toxicity of epsilon-metofluthrin may be enhanced when administered in corn oil, a standard toxicology vehicle. Although it is noted that in the acute neurotoxicity screening study with 5 males and 5 females there were no mortalities at 100 mg/kg bw. Also, in another non-GLP investigative study where

10 females were dosed with 100 mg/kg bw epsilon-metofluthrin in corn oil, only 1/10 females died.

It is noteworthy that in a rat developmental toxicity study (see section 4.11.2), where epsilon-metofluthrin was administered as a gavage bolus dose in corn oil, very low maximum doses were achieved (30 mg/kg bw/day). In contrast, in the multigenerational studies (see section 4.11.1), in which epsilon-metofluthrin was administered via the diet, much higher doses were possible (125-280 mg/kg bw/day), doses that would have been expected to cause significant mortalities if administered as a gavage bolus in corn oil. These observations of a clear difference between corn oil and dietary dosing are consistent with the single oral exposure studies which indicate that epsilon-metofluthrin is more toxic when administered in corn oil.

It is possible there were vehicle dependent differences in toxicokinetics that were revealing an intrinsic hazardous property of epsilon-metofluthrin (acute oral toxicity). There do not appear to be clear methodological reasons to exclude findings from the single oral exposure studies with corn oil vehicle (as it is a standard vehicle). An LD_{50} value was not identified in the acute neurotoxicity study, but given the mortalities (7/20) at 100 mg/kg bw, it was proposed to classify epsilon-metofluthrin as Acute Tox. 3; H301, considering the values of 50 mg/kg bw < ATE ≤ 300 mg/kg bw in Annex I of CLP.

Acute toxicity: dermal

There was no evidence of systemic toxicity or mortalities in rats acutely exposed to doses of up to 2000 mg/kg bw and no classification was proposed.

Comments received during public consultation

One industry organisation and one MSCA disagreed with the classification as Acute Tox. 3 proposed for the oral route, considering that the toxicity data using epsilon-metofluthrin in corn oil does not reflect the intrinsic property of epsilon-metofluthrin and should not be used for classification purposes. The industry organisation also argued that based on comparative toxicokinetic considerations between the oral route and inhalation a classification as Acute Tox. 4 by the oral route should be considered.

Another MSCA supported the proposed classification both for oral route and inhalation.

Assessment and comparison with the classification criteria

Acute toxicity: inhalation

A GLP-compliant study performed according to the OECD TG 402 reports a 10% mortality in Sprague-Dawley rats exposed nose-only to 1 mg/L of a mist aerosol of epsilon-metofluthrin and 100% mortality in rats exposed to 2 mg/L (no mortality at the low dose of 0.5 mg/L). The LC_{50} by inhalation in rats is therefore between 1 and 2 mg/L, which is in the range 1.0 - 5.0 mg/L relevant for classification in category 4 by inhalation.

On this basis, RAC recommends classification as **Acute Tox. 4; H332** for epsilon-metofluthrin.

Acute toxicity: oral

In a guideline-compliant study in which epsilon-metofluthrin was administered undiluted, the LD_{50} was shown to be above the classification threshold of 2000 mg/kg bw. However, single administrations of epsilon-metofluthrin in corn oil have been shown to induce lethality at much lower doses. The neat substance is a liquid with a log K_{OW} of 5.0 and very low water solubility (<1 mg/L) and RAC notes that a lipophilic solvent such as corn oil is indeed appropriate. Although the corresponding studies were not performed for the purpose of LD_{50} estimation they are

considered to provide appropriate information for the discussion of acute toxicity classification by the oral route. Studies with single exposures by the oral route are summarised in the table below.

Summary of studies with a single exposure to epsilon-metofluthrin by the oral route

Type of study	Vehicle	Species	Mortality	Clinical signs	Reference
Acute toxicity (OECD TG 401)	None	Sprague-Dawley rat	2000 mg/kg bw: 1/5 death in females; 0/5 in males. 1000, 1500 mg/kg bw: no deaths	Clinical signs at 2000 mg/kg bw only.	Kunimatsu, 2002a
Acute neurotoxicity (OECD TG 424)	Corn oil	Sprague-Dawley rat	<u>Main study (n=10/sex):</u> 100 mg/kg bw: 7/20 animals dead or sacrificed moribund	No information	York, 2004a
			<u>Range finding (n=5/sex):</u> 20, 50, 100 mg/kg bw: no deaths	Clinical signs (dose levels affected unclear)	
			<u>Subsequent study (n=10 females):</u> 100 mg/kg bw: 1/10 death (22 hr)	100 mg/kg bw: clinical observations in 6/10 females	
Sighting study for micronucleus test	Corn oil	CD-1 mouse	60 mg/kg bw: 2/10 deaths. 12.5, 25, 50 mg/kg bw: no deaths	Clinical signs at 60 mg/kg bw. No detailed information at lower doses.	Odawara, 2002c

The analysis of studies with repeated administration confirms that the mode of administration of epsilon-metofluthrin significantly impacts the dose at which toxicity is exhibited. Mortality was observed in dams exposed to 30 mg/kg bw epsilon-metofluthrin in corn oil by gavage in a prenatal developmental toxicity study. Conversely, no mortality was observed in rats up to 273/285 mg/kg bw in a 28-day study when the substance was administered via the diet. A probable explanation is that exposure to a bolus dose in a lipophilic medium, such as corn oil, facilitates extensive oral absorption of epsilon-metofuthrin. This resulted in the observed higher systemic peak of epsilon-metofluthrin, which is possibly responsible for the neurotoxic effects and death of the animals.

Such toxicity of epsilon-metofluthrin revealed by administration in corn oil reflects an intrinsic property of epsilon-metofluthrin and should be considered for classification. It is consistent with the CLP guidance that states in section 3.1.2.3.2 that "If there are different LD_{50} values from tests using different vehicles (e.g. water vs. corn oil or neat substance vs. corn oil), generally the lowest valid value would be the basis for classification." Besides, due to its poor water solubility, if any dilution of the substance is needed, such as in commercial products, a lipophilic solvent would be required. Although corn oil may not be used as a solvent in products containing epsilon-metofluthrin, it is nevertheless considered to be a relevant solvent in these studies.

RAC notes, however, that none of the experiments performed using corn oil as a vehicle provide a true estimation of the LD_{50}. In rats, mortality was observed at 100 mg/kg: in the different experiments, the mortality rates at this dose level ranged from 0% up to 35% in the main study (7/20 animals). Overall, it represents a mortality rate of 20% (8/40 animals). In a sighting study for a micronucleus test, a mortality of 20% was consistently observed in mice at 60 mg/kg.

The LD_{50} of epsilon-metofluthrin in corn oil is therefore not known with certainty from experimental data. RAC notes that the dose-response relationship for mortality is likely to follow a steep slope with acute toxicity by inhalation showing 10% of mortality (assumed to be secondary to systemic effects) at 1 mg/L and 100% at the next higher dose of 2 mg/L. This supports the LD_{50} by the oral route most likely being below 300 mg/kg. Further comparison of the results obtained by inhalation and by the oral route, assuming 100% of absorption for both routes (see table below), shows that mortality is generally observed at lower estimated internal doses by gavage in corn oil than by inhalation, although a first-pass detoxification may occur via the oral route. This observation may reflect that the relative absorption is indeed greater and/or more rapid by gavage in corn oil than by inhalation and that as a result higher dose or peak level of epsilon-metofluthrin are reached. In contrast, it was argued during public consultation that mortality is observed earlier after the start of exposure by inhalation, indicating that oral absorption into the systemic circulation is slower'compared to inhalation. This would seem consistent with the estimated systemic exposure by inhalation representing a more severe scenario for acute toxicity than by the oral route. As the extent and rate of systemic exposure are greater by inhalation, a lethal level is anticipated to be reached more quickly.

Comparison of estimated internal doses assuming 100% of absorption in acute toxicity studies performed with Sprague-Dawley rats by inhalation or by gavage in corn oil

GAVAGE IN CORN OIL		
Estimated exposure	Mortality	Time of death
50 mg/kg	0/10	-
100 mg/kg	8/40 (20%)	Ethical sacrifice of most animals 4-7 h post-dosing. 1 death 22 h after dosing

INHALATION		
Estimated exposure	Mortality	Time of death
96 mg/kg	0/10	-
192 mg/kg	1/10 (10%)	No data
384 mg/kg	10/10 (100%)	During or immediately after exposure

Finally, acute toxicity data in rats exposed to epsilon-metofluthrin in corn oil via theoral route have been identified for two relevant structurally related pyrethroids. Epsilon-momfluorothrin (CAS 1065124-65-3) has an LD_{50} that is over 2000 mg/kg bw in males and between 300 and 2000 mg/kg bw in females. The dose response seems to be steep as no mortality is observed at 300 mg/kg bw and yet 70% mortality is observed at the next dose level of 2000 mg/kg bw. However, the large interval between the two doses does not allow a clear conclusion on the slope of the dose-response curve. In contrast, tefluthrin (CAS 79538-32-2) has an LD_{50} of 21.8 mg/kg bw in males and 34.6 mg/kg bw in females. Mortality was 0% at 10 mg/kg bw and 100% at 47 mg/kg bw, which tends to show a steep dose-response relationship. These data tend to suggest that the acute oral LD_{50} of these types of pyrethroids may vary from high to low acute toxicity depending on the substance and that a steep dose-response relationship may be expected for these compounds.

Overall, the comparison of data by inhalation and gavage in corn oil supports (considering a 20% death rate at 100 mg/kg bw by gavage in corn oil and considering a higher relative toxicity than by inhalation) more than 50% of mortality being expected at the upper limit of 300 mg/kg bw for Acute Tox 3 classification.

On this basis, RAC concludes that classification as **Acute Tox. 3; H301** is warranted for epsilon-metofluthrin by the oral route.

Acute toxicity: dermal

No mortality or signs of toxicity were observed in a GLP compliant OECD TG 402 study in rats up to 2000 mg/kg.

Considering an LD_{50} > 2000 mg/kg bw in rat, **no classification is recommended by RAC for acute toxicity by dermal route**

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

Summary of the Dossier Submitter's proposal

Summary

Clinical signs of neurotoxicity were noted in a standard acute oral dosing study, at the highest dose of 2000 mg/kg, a dose that caused a single mortality. In a single exposure study (acute neurotoxicity) in which rats were dosed with epsilon-metofluthrin in corn oil, clinical signs of toxicity such as tremors, twitches, tachypnoea (females), excess salivation, prostrate, lost righting reflex, clonic convulsions, tonic extensor convulsions, and hyperpnoea (males) were only observed at the top dose of 100 mg/kg. At this dose, 2/10 males and 1/10 females were found dead and a further 4 females were sacrificed *in extremis*. In the range finding study, in which epsilon-metofluthrin was administered to 5 male and 5 female rats at doses of 0, 20, 50 and 100 mg/kg, there were no deaths. However, whole body twitches were observed in 2 males and 4 females at the 2 and 3 hour time points only.

In a non-GLP compliant study subsequently conducted to evaluate the toxicity of epsilon-metofluthrin, 10 females were administered a single dose of 100 mg/kg bw epsilon-metofluthrin in corn oil, under the same conditions as the main acute neurotoxicity study. 1/10 females died 22 hours after dosing. This animal exhibited intermittent whole body twitches at approximately 4.5 hours after dosing, hyperactivity to sound at approximately 6 hours post dosing and continuous whole body tremors at approximately 6.5 hours after dosing. All other rats survived until terminal sacrifice and clinical observations in these animals included whole body tremors (6/10), whole body twitches (5/10) and hyperactivity to sound (6/10). These effects were seen from the 3.5 hour observation point and all surviving animals appeared normal the day after dosing. Tremors were noted in mice administered epsilon-metofluthrin in corn oil at a dose of 60 mg/kg, a dose that caused mortalities (2/10).

The Dossier Submitter noted that although epsilon-metofluthrin induced clinical signs of neurotoxicity after single exposures, it was only at doses that caused mortalities. It was therefore argued that classification for STOT SE is not warranted.

Comments received during public consultation

One industrial organisation supported no classification for STOT SE.

Assessment and comparison with the classification criteria

Oral route

By the oral route, serious signs of neurotoxicity in the acute toxicity studies were observed at doses below the guidance value for classification for STOT-SE 1 (< 300 mg/kg bw via oral

exposure). Signs of neurotoxicity were also observed in several repeated dose toxicity studies by the oral route as summarised in Table below. Although the exact onset of tremors is not reported, available information indicates that these effects are acute in nature. It is also supported by the absence of histopathological or functional long term findings investigated through detailed examination and FOB in the 90-day neurotoxicity study in rats.

Clinical signs related to neurotoxicity in repeat dose toxicty (RDT) studies by the oral route at doses ≤ guidance value for STOT SE 1 (i.e. 300 mg/kg bw)

Study	**Clinical signs related to neurotoxicity in RDT studies at doses ≤ classif. threshold (STOT SE 1 = 300 mg/kg bw)**
RAT - diet	
28-day	285/273 mg/kg bw: tremor (27/36)
90-day	-
90-day neurotoxicity	179/206 mg/kg bw: tremors (5/24) and twitches (3/24)
26-week	165/191 mg/kg bw: tremors in all animals during week 1, declining rapidly until no tremor in week 4
2-year	-
MOUSE – diet	
90-day	-
78-week	-
DOG - capsules	
90-day	100 mg/kg bw: tremor (10/12) 2-6 h post-admin. (vomiting from 30 mg/kg)
1-year	≥ 30 mg/kg bw: tremor (7/8) 2-6 h post-administration (and vomiting)

However, the acute neurotoxic effects observed both in acute and RDT studies generally occurred at doses causing lethality after single exposure that justify the classification for Acute Toxicity. Classification for STOT SE by the oral route is therefore not appropriate.

Inhalation

By inhalation, serious signs of neurotoxicity (tail tremor during exposure, tremor, hypersensitivity, ataxic gait, tiptoe gait and clonic convulsion post-exposure) were observed in the acute study. Although the exact severity and incidence of each finding is not known, these effects were reported in all exposure groups, including the lowest dose of 0.5 mg/L that did not induce mortality.

In addition, tremor was observed in the 28-day study at the dose level of 0.2 mg/L (4h/day) and is considered as an acute (repeated) effect. Mortality was also observed at this dose level but comparing with absence of mortality at 0.5 mg/L in the acute toxicity study and that deaths occured after multiple exposure (day 3 to day 27), they are not considered as an acute effect (see also section on STOT RE). It is noted that tremor is a transient effect of low adversity and its relevance to fulfil the severity criteria for STOT SE is unclear. However, it supports information from the acute toxicity studies that reported more serious neurotoxic clinical signs at the non lethal dose of 0.5 mg/L.

These findings were observed in both studies at doses below the threshold of 1 mg/L (4h/day) for classification for STOT SE in category 1.

Overall, on the basis of data by the inhalation route, classification as **STOT SE 1 (nervous system)** is justified.

The route of exposure is not specified as no information is available through the dermal route.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

According to the Dossier Submitter, epsilon-metofluthrin when tested on rabbits according to OECD TG 404 under GLP, did not produce skin effects meeting the criteria for classification as a skin irritant.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

In the skin irritation study (Nakamura, 2001a), carried out under GLP according to OECD TG 404, epsilon-metofluthrin caused mild transient erythema but the mean scores did not meet the CLP criteria for classification as a skin irritant (i.e., all scores were < 2.3 for erythema and oedema in all animals). Therefore, in the opinion of RAC, epsilon-metofluthrin **does not warrant classification as skin irritant**.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

According to the Dossier Submitter, epsilon-metofluthrin tested on rabbits according to OECD TG 405 under GLP did not produced eye irritation effects meeting the CLP criteria for classification as an eye irritant.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

In the eye irritation study (Nakamura, 2001a; A.6.1.4 Doc IIIA) carried out on rabbits according to OECD TG 405 under GLP, epsilon-metofluthrin did not result ineye irritation effects (*i.e.* all scores were 0 in all three animals). Therefore, in the opinion of RAC, epsilon-metofluthrin **does not warrant classification as an eye irritant**.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

The potential of epsilon-metofluthrin to cause respiratory sensitisation was not investigated directly.

However, given that epsilon-metofluthrin does not have skin sensitisation properties and the sub-acute inhalation study gave no indication of respiratory sensitisation, epsilon-metofluthrin is considered unlikely to be a respiratory sensitiser. Therefore no classification is proposed.

Comments received during public consultation

No comments during public consultation

Assessment and comparison with the classification criteria

In the opinion of RAC there are no data suggesting that epsilon-metofluthrin may cause respiratory sensitisation.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

Based on results of a standard guinea pig maximisation test epsilon-metofluthrin is not a skin sensitiser.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

In the standard maximisation test (Nakamura, 2002b; A.6.1.5 Doc IIIA) carried out on guinea pigs according to OECD TG 406 under GLP, epsilon-metofluthrin did not cause positive skin reactions in the challenge test in any animal. Therefore, in the opinion of RAC, epsilon-metofluthrin **does not warrant classification as skin sensitiser**.

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

Summary of the Dossier Submitter's proposal

Oral

An increased incidence of hepatocellular hypertrophy was observed in rats following repeated oral exposure to epsilon-metofluthrin. In a standard 90 day dietary study, hepatocyte hypertrophy accompanied by hepatocyte basophilia was observed at doses of 21-22 mg/kg bw/day and above. Marked liver weight increases (around 20%) were only observed at doses of

70-73 mg/kg bw/day and above. There was no evidence of functional disturbance at any dose level. Overall, hepatocyte hypertrophy in the absence of marked liver weight increases or perturbation of liver function does not support classification for repeated dose toxicity. No classification is proposed by the DS.

Dermal

No evidence of systemic toxicity was observed in a standard 90 day dermal toxicity study in rats, at doses of up to 1000 mg/kg bw/day, the highest dose tested. Therefore no classification is proposed by the DS for repeated dermal toxicity.

Inhalation

The classification cut-off for STOT RE category 2 for effects in rat 90 day inhalation studies is 0.02-0.2 mg/L/6h day. This scales to 0.06-0.6 mg/L/6h day for an equivalent 28-day study (using Haber's rule). Following repeated inhalation exposure of rats for 28 days to an epsilon-metofluthrin aerosol, mortality and tremors were observed at a dose 0.2 mg/L/4h day, the highest concentration tested. As mortalities occurred throughout this study and are not considered to be a manifestation of the acute inhalation toxicity of epsilon-metofluthrin, classification with STOT RE Category 2; H373 (inhalation) is proposed by the DS.

Comments received during public consultation

One MSCA supported the classification as STOT RE 2 proposed for inhalation. One industry organisation disagreed considering that the mortality observed is a manifestation of acute toxicity.

Assessment and comparison with the classification criteria

Oral

Metofluthrin has been tested by the oral route in rats (28-day study, 90-day studies in two strains, 26-week and 2-year studies), mice (90-day and 78-week studies) and dogs (90-day and 1-year). Epsilon-metofluthrin has been administered through the diet in all studies with rats and mice and in capsules in dogs.

In rats and mice, the liver is the main target organ. Changes observed at the doses relevant for classification consisted of increased plasma cholesterol and phospholipids, increased liver weight, hepatocellular hypertrophy and basophilia in rats. In some studies, dark liver was also reported in rats. Similar effects occurred in mice at doses above the classification threshold and this also includes hepatocellular degeneration/necrosis in the highest dose group of the mouse 90-day study. In rats, hepatocellular steatosis, variation in bilirubin, increased plasma triglycerides and γ-GT, and cell foci were observed at doses above the classification threshold.

Kidneys were also identified as target organs in rats at the highest dose in the 2-year study that exceeds the classification threshold. The effect consisted of an increase in lipofuscin deposition, increases in incidence of tubular casts and interstitial fibrosis, and tubular vacuolation. No kidney effect was observed at lower doses.

Tremors were observed in rats and dogs in several studies at doses above (90 day study and 26 week study in rats and 1 year study in dogs) or at the level (285/273 mg/kg bw in the 28 day study in rats and 100 mg/kg bw in the 90 day study in dogs) of the classification threshold. In both dog studies an increased incidence in vomiting post-administration was also noted. In the 90-day study in Sprague-Dawley rats, the death of 1 female rat was also reported at a dose exceeding the classification threshold. In mice, clinical signs indicating neurotoxicity were not reported but epsilon-metofluthrin induced mortality at a dose largely exceeding the classification threshold.

Overall, the effects observed in the liver of rats at doses relevant for classification (increased plasma cholesterol and phospholipids, increased liver weight, hepatocellular hypertrophy and basophilia) are not considered sufficiently severe to indicate clear organ dysfunction and do not justify classification as STOT RE by the oral route. Tremors observed in rats and dogs at the limit of the classification threshold are indicative of neurotoxicity. However, although detailed information is not available on the timing of the onset of the effects, it is observed in all animals in the first week of the 26 week study before declining until week 4 after which no effect was observed. A similar pattern was observed in the dog studies 2 to 6h after exposure. In addition, no histopathological findings in the nervous system after detailed examination and no functional findings in the FOB were reported. Altogether, the information therefore points toward an acute effect, as was considered to be the case for the STOT SE hazard class. No STOT RE classification is therefore warranted by the oral route.

Dermal

By the dermal route, epsilon-metofluthrin has been tested in a 90 day study in rats. Treatment-related effects occurred only at the highest dose of 1000 mg/kg bw in females and consisted of slight squamous cell hyperplasia at the application site and mortality of 2 females, with tremors in one of these females prior to death. No effects were observed at doses relevant for classification and no classification is warranted for STOT RE by the dermal route.

Inhalation

Epsilon-metofluthrin has been tested in a 28 day inhalation study in rats. Rats were exposed to epsilon-metofluthrin as an aerosol for 4 hours per day, 7 days a week for 4 weeks. No treatment related changes were observed at concentrations of 0.01, 0.05 and 0.1 mg/L. At the highest dose of 0.2 mg/L tremors were observed during or immediately after exposures in 5/10 males and 5/10 females. However, this sign of neurotoxicity is considered to be of an acute nature and relevant for STOT SE consideration but not STOT RE.

Three females died, one each on days 3, 4 and 5, and seven males died on days 4 (3 deaths), 9, 19, 25 and 27. No histopathological changes were observed locally or systemically. This effective dose is relevant for classification STOT RE 2 (range of 0.06-0.6 mg/L/6 h/d for a 28 day study). After acute exposure, the lowest dose that induced mortality was 1 mg/L (1/10 deaths) whereas no deaths were reported at 0.5 mg/L. Deaths after repeated exposure therefore occur at lower levels. Besides, deaths are distributed throughout the study and are therefore considered distinct from the acute lethal effect. One member expressed a minority opinion arguing that the deaths were covered by the acute toxicity and that STOT RE was not warranted; their grounds are published along with this opinion.

On the basis of the mortality observed by inhalation after repeated exposure in rats at the concentration of 0.2 mg/L, RAC concludes that classification as **STOT RE 2; H373** is warranted without specifying a target organ.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The genotoxicity of epsilon-metofluthrin has been investigated, both *in vitro* (bacterial gene mutation, mammalian cell gene mutation and chromosomal aberration) and *in vivo* (mouse micronucleus), giving negative results in all tests. Although no reduction in PCE/NCE ratio was observed, there are data from toxicokinetic studies indicating that following oral dosing, epsilon-metofluthrin is distributed to the bone marrow.

As epsilon-metofluthrin tested negative *in vitro* and *in vivo*, and there are no human data available, classification for germ cell mutagenicity is not justified.

Comments received during public consultation

Two individuals supported the absence of genotoxic potential of epsilon-metofluthrin.

Assessment and comparison with the classification criteria

The outcome of the *in vivo* bone marrow micronucleus test performed according to OECD TG 474 was negative. It is supported by negative results in *in vitro* studies investigating mutation in bacteria, chromosomal aberrations and gene mutation in mammalian cells, and performed according to appropriate guidelines. RAC agrees that **classification for mutagenicity is not warranted** for epsilon-metofluthrin.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

Lifetime dietary administration of epsilon-metofluthrin induced a clear increase in liver tumours (adenomas and carcinomas) in male and female rats. The incidence of liver carcinomas in males exceeded both the concurrent and historical control incidence at doses of 38 mg/kg bw/day and above. In both sexes, administered the highest dose of 78-96 mg/kg bw/day, terminal body weights were decreased compared to controls but the findings were not considered to indicate that the MTD had been exceeded. It was also noted that the observed tumours were late onset and occurred at a single site. No treatment-related increases in tumour incidence were observed following lifetime dietary administration in mice.

The findings from an extensive and well-conducted set of short-term *in vitro* and *in vivo* assays have indicated that epsilon-metofluthrin is unlikely to have acted as a genotoxic carcinogen. In contrast, there are mechanistic data available that point towards a non-genotoxic mechanism of carcinogenesis, driven initially by activation of the nuclear constitutive androstane receptor (CAR) and later by a proliferative response in the liver.

The mode of action for the induction of rat liver tumours has been postulated to involve the following sequence of events:

1. Nuclear membrane receptor activation (CAR)
2. Altered gene expression specific to CAR activation (CYP 2B induction)
3. Liver hypertrophy
4. Increased hepatocellular proliferation
5. Clonal expansion to generate altered liver cell foci
6. Increased adenoma/carcinoma.

The key events are considered to be CAR activation, increased cell proliferation and the eventual induction of altered hepatic foci. Events, such as Phase 1 enzyme induction and decreased apoptosis are regarded as associative rather than key events.

The mechanistic studies indicated that epsilon-metofluthrin is a CAR activator, inducing CYP 2B1/2 and hepatocellular proliferation, *in vivo*, in rats.

Other modes of action, such as PPARα, AhR or PXR were not supported by the available mechanistic data. The most prominent finding from the mechanistic studies was a species difference in the potential of CAR activators to induce proliferation in primary hepatocyte cultures *in vitro*. Human hepatocytes were found to be completely unresponsive to mitogenic stimulation with epsilon-metofluthrin and phenobarbitone in two separate investigations using male and female donors. In contrast, rat hepatocytes responded with increased replicative DNA synthesis. Both human and rat hepatocytes responded to growth factor-stimulated mitogenesis, demonstrating that they were responsive preparations.

Overall, the available data indicate that epsilon-metofluthrin induced liver tumours in rats against a background of CAR activation and hepatocellular proliferation. The *in vitro* data confirm that human hepatocytes have the capacity to respond to CAR activators, but suggest the response is limited to hypertrophy and not replicative DNA synthesis and hyperplasia. The finding that human hepatocytes are refractory to CAR-induced mitogenesis, a key event in the tumour progression pathway, gives reassurance that the rat liver tumours induced by epsilon-metofluthrin, have limited or no relevance for human health.

The DS concluded that classification as Carc. 1B is not justified as the animal data are limited rather than sufficient due to tumour incidence being restricted to one species (rats), in a single tissue with no evidence of a genotoxic mode of action. Taking into account the *in vitro* and *in vivo* mechanistic data discussed above, Carc. 2 is not justified, as the data indicate that the liver tumours caused by epsilon-metofluthrin in rats are induced by CAR activation, a mechanism which is not relevant for human health. Therefore, no classification was proposed.

Comments received during public consultation

Two individuals submitted comments that supported the mode of action of epsilon-metofluthrin hepatocarcinogenicity mediated through CAR activation and the absence of its relevance for humans, requiring no classification. This was further supported by an industry organisation.

One MSCA suggested a classification in category 2 considering that the absence of liver tumours in mice is inconsistent with the mode of action through CAR activation and that CAR-related tumours are not explicitly considered as not relevant for humans according to CLP guidance.

Assessment and comparison with the classification criteria

Carcinogenicity of epsilon-metofluthrin has been evaluated in a 2 year study in Wistar rats and in a 78 week study in CD-1 mice. In both studies exposure was through the diet.

In rats at the highest dose, statistically significant increases in hepatocellular adenomas and in carcinomas was observed in males and females (table below) and the incidence of both adenomas and carcinomas were above contemporary historical control data in both sexes. At 38/47 mg/kg bw, the incidence of adenomas in males and of carcinomas in males and females were also above historical control data. When combined, the incidence of adenomas and carcinomas in males was statistically significant.

Summary of hepatic non-neoplastic lesions and neoplastic findings in the rat two year study

Doses in M/F (mg/kg bw)		0	0.8/1.0 (20 ppm)	8/10 (200 ppm)	38/47 (900 ppm)	78/96 (1800 ppm)	HCD
Hepatocellular hypertrophy	M	-	-	-	-	26%	
	F	-	-	-	24%	-	
Clear cell foci	M	-	-	-	-	-	
	F	-	-	-	-	64%	
Eosinophilic foci	M	-	-	-	-	20%	

	F	-	-	-	-	-	
Mixed cell foci	M	-	-	-	18%	-	
	F	-	-	-	-	24%	
Hepatocellular adenomas	M	2%	2%	6%	10%	12%*	0-8%
	F	2%	2%	0	6%	14%*	0-10%
Hepatocellular carcinomas	M	0	0	0	6%	16%*	0-2%
	F	0	4%	2%	4%	14%*	
Hepatocellular combined	M	2%	2%	6%	16%*	24%*	0-10%
	F	2%	6%	2%	10%	24%*	0-12%

*statistically significant; HCD: contemporary historical control data in the test laboratory; - : no specific findings reported

In mice, the highest dose of 2500 ppm was reduced to 1750 ppm on the second week of exposure due to mortality. The corresponding estimated dose was 209 mg/kg bw in males and 277 mg/kg bw in females. In this study, no treatment-related change in tumour incidence was observed.

A number of *in vitro* and *in vivo* studies have been conducted to address the MoA responsible for the liver tumour formation in rats and evaluated according to the 2006 IPCS framework for human relevance (Boobis, 2006). These studies are briefly summarised below (for more details see section 4.10.3 of the Background Document). Carcinogenicity through CAR activation has been postulated and in several studies the prototypical CAR activator phenobarbital (PB) was included as a positive control.

In vitro study with rat hepatocytes to investigate gene expression specific to CAR activation (Deguchi, 2009)

Primary rat hepatocytes (male, Wistar) transfected with CAR siRNA (short interfering RNA specific to CAR, used to block transcription of CAR mRNA and decrease the amount of functional CAR) or control siRNA (negative control) were exposed to 50 µM epsilon-metofluthrin or to 50 µM PB. Induction of CAR mRNA in hepatocytes transfected with CAR siRNA was reduced by 34-37% in presence of epsilon-metofluthrin or PB compared to negative control. It resulted in a reduced induction of CYP2B1 mRNA levels to 32% and 21% of controls with epsilon-metofluthrin and PB, respectively. This indicates that mainly CAR activation is involved in the induction of CYP2B1 mRNA by epsilon-metofluthrin.

In vitro study with rat and human hepatocytes to investigate CYP2B induction and replicative DNA synthesis (Hirose, 2009)

Primary hepatocytes from male Wistar rats and from one human female donor were cultured with 50 µM epsilon-metofluthrin or 50 µM PB. Expression of mRNA for CYP2B1 in rat hepatocytes was increased by 3.4 fold by epsilon-metofluthrin and 3.7 fold by PB and expression of mRNA for human orthologue CYP2B6 in human hepatocytes was increased by 2.4 fold by epsilon-metofluthrin and 16.0 fold by PB. CYP2B enzymatic specific activity was significantly increased in rat hepatocytes by epsilon-metofluthrin and PB (25 and 37%, respectively) and it was increased in human hepatocytes by epsilon-metofluthrin and PB (25 and 80%, respectively) although the increase induced by epsilon-metofluthrin was not statistically significant.

In a second part of the study, effects on replicative DNA synthesis was investigated by BrdU labelling in primary rat hepatocytes and human hepatocytes from two female donors. Replicative DNA synthesis was statistically significantly increased in rat hepatocytes treated with epsilon-metofluthrin at concentrations of 10 µM and above (by around 50% compared to controls), and in PB treated rat hepatocytes at 100 and 500 µM (by around 20%) but not at 1000 µm. No effect on replicative DNA was observed in human hepatocytes when tested with either epsilon-metofluthrin or PB, although a clear positive dose-response relationship was obtained when incubated with hepatocyte growth factor, showing responsiveness of the experimental system.

In vitro study with human hepatocytes to investigate CYP2B induction and replicative DNA synthesis (Yamada, 2012)

The study by Hirose (2009) described above was replicated using human hepatocytes cultures from 2 male and 2 female donors. Expression of mRNA for CYP2B6 was increased by 1.9 fold by epsilon-metofluthrin and 5 fold by PB (1000 µM each). No effect on replicative DNA was observed in human hepatocytes when tested with either epsilon-metofluthrin or PB, although a clear positive dose-response was obtained when incubated with hepatocyte growth factor.

In vivo rat studies (Deguchi, 2009)

A series of studies were performed in Wistar rats exposed through diet at doses including the dose levels of 900 and 1800 ppm that resulted in increased incidences of liver adenoma and carcinoma in the 2-year study. For comparison, rats were also dosed with 1000 ppm NaPB in the diet.

The findings of a first 7 days study are presented in table below.

Main findings of the 1st study in Deguchi (2009)

	200		900		1800		3600		Na PB	
	M	F	M	F	M	F	M	F	M	F
Number examined	5	5	5	5	5	5	7	7	5	5
Tremors	0	0	0	0	0	0	3/7	2/7	0	0
Death	0	0	0	0	0	0	0	2/7	0	0
Rel. liver wt	0	0	+6%	+8%	**+13%***	**+12%***	+6%	**+19%***	**+19%***	**+22%***
Liver enlarg.	0	0	0	0	1	0	1	2	3	3
Dark liver	0	0	0	0	1	2	1	3	**4***	3
Hepatocell. hypertrophy	0	0	0	0	1	1	2	2	**4***	3
Hepatocell. SER changes	ND	ND	ND	ND	ND	ND	2/2	2/2	2/2	2/2
CYP 2B1/2 mRNA expr.	x1.6	x1.9	x2.9	**x5.4***	**x6.2***	**x12.9***	**x10.5***	**x17.9***	**x125.3***	**x64.4***
CYP 2B protein level	x1.3	x1.2	x1.5	x1.4	x1.8	x1.7	**x2.4***	**x2.1***	**x23.7***	**x8.1***
BrdU labelling index	x1.2	x0.6	**x2***	x0.8	**x2.2***	x1.3	x0.5	x1.2	**x3.5****	**x1.7***

ND: no data; ***: statistically significant**

Liver gene profiling was also performed in 3 animals in controls, the 1800 ppm test group and the Na PB group. Epsilon-metofluthrin and Na PB altered expression of genes with a similar profile. Most of the genes that were upregulated by epsilon-metofluthrin were metabolic enzymes including glutathione- S-transferase, CYPs and UDPglucosyltransferase (UGTs). These genes were also upregulated by NaPB with greater potency. Induction of CYP3A (induced through PXR) was also investigated. With epsilon-metofluthrin the mRNA for CYP3A1was increased statistically significantly at 3600 ppm in males and at 1800 and 3600 ppm in females but CYP3A2 mRNA was not increased at any of these dose levels. There was no increase in CYP3A protein levels at any of these doses. Hepatic activities of testosterone 6b-hydroxylase (a CYP3A marker) were increased in females treated with epsilon-metofluthrin 3600 ppm. The study included a 7 day recovery group and all changes were reversible on cessation of treatment.

An additional study investigating the effects after 7 or 14 days of exposure was performed with a epsilon-metofluthrin concentration of 2700 ppm. Statistically significant increases in relative

liver weight and expression of CYP 2B1 mRNA were observed after 7 and 14 days of exposure. A significant increase of the BrdU labelling index (numeric data not available) was observed after 7 days but not after 14 days.

A final study investigated additional cellular events after a 7 day exposure. Indications of a decreased gap junctional intercellular communication was observed from 1800 ppm. No effect of epsilon-metofluthrin was observed on lipid peroxidation or apoptosis. An increase in reduced glutathione may however indicate some oxidative stress. Results are summarised in table below.

Main findings of the 3rd study in Deguchi (2009)

	200		900		1800		3600		Na PB	
	M	F	M	F	M	F	M	F	M	F
Nber examined	8	8	8	8	8	8	8	**8**	8	8
Tremors	0	0	0	0	0	0	2	**2**	0	0
Rel. liver wt	-1%	+3%	+3%	+7%	**+12%***	+9%	**+15%***	**+26%***	**+19%***	**+25%***
Liver enlarg.	0	0	0	0	1	0	1	**3**	**6***	4
Dark liver	0	0	0	0	1	0	1	**1**	**6***	2
Hepatocell. hypertrophy	0	0	0	0	1	0	2	**1**	**8***	**8***
Hepatic GJIC capacity	x0.94	x0.99	x0.80	x0.85	**x0.66***	**x0.70***	x0.81	**x0.74***	**x0.52***	**x0.47***
Lipid peroxidation	x0.99	x0.99	x1.07	x0.99	x0.99	x0.93	x1.01	x0.89	x1.03	**x0.81***
Reduced GSH	x1.27	x1.25	**x1.32***	x1.25	**x1.54***	**x1.69***	**x1.71***	**x2.13***	x1.02	**x1.63***
Apoptosis	x1.07	x1.18	x1.08	x0.92	x1.11	x0.97	x0.93	x1.16	**x0.63***	x1.07

GJIC :gap junctional intercellular communication; *** : statistically significant**

In these studies, significant increases in relative liver weight and liver hypertrophy in some animals were consistently observed from 1800 ppm. Tremors and occasionally death were reported at 3600 ppm.

In vivo mouse study (Deguchi, 2008)

Treatment of CD-1 mice with epsilon-metofluthrin for two weeks produced some increases in liver weight, centrilobular hepatocyte hypertrophy and induction of the CYP2B marker enzyme activity 7-pentoxyresorufin O-depentylase in both sexes. The effects were significant from 1000 ppm in males and 1750 ppm in females and were greater in male than in female mice.

Additional information provided during and after public consultation

RAC was informed in a comment submitted during the public consultation that the effect of epsilon-metofluthrin on replicative DNA synthesis has been investigated *in vivo* in human hepatocytes using chimeric mice and the corresponding report was provided to RAC after the initial public consultation (Yamada, 2015).

In three separate studies, chimeric mice with human hepatocytes from different donors were treated with 1800 ppm epsilon-metofluthrin in the diet for 7 days. Treatment with epsilon-metofluthrin did not result in any increase in replicative DNA synthesis. However, as a positive control, replicative DNA synthesis in human hepatocytes was increased when the chimeric mice were treated with epidermal growth factor, indicating that they had the potential to respond.

In the interests of transparency, this report was made the subject of a further targeted public consultation.. Comments were received from one MSCA that raised uncertainties linked to this

experimental model. In particular, the potential influence of the rodent environment on human hepatocytes, the pre-existing liver disease in the mouse strain, metabolic differences due to the larger size of the liver in humans and the immaturity of human hepatocytes coming from young children were mentioned as potential issues impacting on the reliability of this model. It was also noted that CYP2B were only weakly induced. Overall, the MSCA was hesitant to draw conclusions on the MoA of epsilon-metofluthrin on the basis of this study.

It was noted above that metofluthrin induces benign and malignant liver tumours in male and female rats but not in mice.

From the mutagenicity data it can be concluded that epsilon-metofluthrin is not genotoxic. Hence, a non-genotoxic MoA is plausible. RAC agrees with the DS that the known non-genotoxic MoAs behind liver tumour formation in rodents are not supported by observations in general toxicity studies: cytotoxicity (necrosis not observed in rats; in mice only at a high dose), no evidence of hormonal perturbation and no evidence of porphyria.

The results of the mechanistic studies further suggest that epsilon-metofluthrin does not act via activation of PPARα as there was no evidence for peroxisome proliferation upon electron microscopy *in vivo*. Although an increased expression of CYP3A1 mRNA has been identified *in vivo* it has not been observed with CYP3A2 mRNA or CYP3A protein level and it does not provide the induction profile that is expected following pregnane x receptor (PXR) activation. Gene expression profiling analysis studies carried out by Yamada *et al* (2006) showed that there were no marked alterations in either PPARα or aryl hydrocarbon receptor (AhR) signaling (no induction of CYP1A1, 1A2, 1B1, 4A1, 4A2 or 4A3).

The evidence available in relation to a CAR-mediated mode of action for liver tumour formation in rats, as well as corresponding evidence in mice and humans are summarised in table below.

Summary of evidence for the different key and associative events of a CAR-mediated induction of liver tumours in rats and corresponding evidence in mice and humans.

Key (K) and Associative (A) Event	Evidence in Rats	Evidence in Mice	Evidence in Humans
Activation of CAR (K)	Experimental evidence *in vitro* using CAR siRNA that CYP2B1 induction is mediated through CAR activation (Deguchi, 2009).	No data (indirect evidence from CYP2B induction)	No data (indirect evidence from CYP2B induction)
Induction of CYP2B (A)	Direct experimental evidence *in vivo* (Deguchi, 2009) and *in vitro* in cultured hepatocytes (Hirose, 2009; Deguchi 2009)	Experimental evidence *in vivo* (Deguchi 2008) of induction of CYP2B activity.	Direct experimental *in vitro* in cultured hepatocytes (Deguchi, 2009; Yamada, 2012)
Hypertrophy (A)	Direct experimental evidence *in vivo* from general toxicity and mechanistic studies	Direct experimental evidence *in vivo* from general toxicity and mechanistic studies	No data
Increased hepatocellular proliferation (K)	Direct experimental evidence *in vivo* (Deguchi, 2009) and *in vitro* in cultured hepatocytes (Hirose, 2009)	No data	Absence of proliferation observed *in vitro* in cultured hepatocytes (Deguchi 2009, Yamada 2012) and *in vivo* in chimeric mice with humanised hepatocytes (Yamada, 2015)
Altered hepatic foci (K)	Direct experimental evidence *in vivo* (2-year study of Schmid, 2005c)	No findings in the 78-week study (Schmid, 2005d)	No data
Liver tumours (K)	Observed *in vivo* (2-year study of Schmid, 2005c)	No findings in the 78-week study (Schmid, 2005d)	No data
Inhibition of gap junctional intercellular communication (A)	Decrease observed *in vivo* (Deguchi, 2009)	No data	No data
Decreased Apoptosis (A)	No significant effect (Deguchi, 2009)	No data	No data

RAC agrees with the DS that CAR activation is the most plausible mechanism behind the liver tumour formation in rats, given the evidence presented for the key events and some of the associative events in this MoA but also with respect to the dose-response relationship and temporal association. The *in vitro* study with rat hepatocytes transfected with CAR siRNA showed that CAR activation is involved in the induction of CYP2B1/2 mRNA by epsilon-metofluthrin (key event 1). The *in vivo* MoA studies in rats consistently showed CYP2B induction, i.e. increased CYP2B1/2 mRNA expression (key event 2, also shown *in vitro* in rat hepatocytes) and increased CYP2B activity (associative event). Electron microscopy further revealed increased SER from 1800 ppm in the 7-day MoA studies, which is characteristic of enzyme inducers. Induction of CYP2B was also identified in the mice.

Increased liver weights and increased incidences of hepatocellular hypertrophy (associative event) were observed in all toxicity (short- and long-term) and MoA studies in rats as well as in mice.

Evidence for increased cell proliferation (key event 3) was provided in an *in vitro* study with rat hepatocytes (Hirose 2009). *In vivo*, a slight but significant increase was observed after 7 days only in males at 900 ppm and 1800 ppm but not at 3600 ppm (Deguchi, 2009 – 1st study) and confirmed in male rats at 2700 ppm after 7 days but not after 14 days (2nd study). Transient stimulation of cell proliferation by epsilon-metofluthrin is similar to what is known for PB. The overall cell proliferation will still be enhanced due to the early increase in the number of hepatocytes.

In chronic studies, these events ultimately resulted in increased incidences of eosinophilic foci (key event 4) and liver tumours (key event 5) in rats, but not in mice. This seems inconsistent, as mice generally appear more susceptible than rats to liver tumour formation by CAR activators. However, for some CYP2B enzyme inducers which appear to have a similar MoA for liver tumour formation to PB, such as pyrethrins and momfluorothrin, liver tumours have been observed in rats but not in mice (Elcombe *et al*., 2014). Momfluorothrin[1] is a close structural analogue to epsilon-metofluthrin. Comparison of the magnitude of the effect on relative liver weight in the 90-day studies also showed that effects in mice were less pronounced than in rats possibly due to toxicokinetic differences and this may explain the difference in intra-species sensitivity. However, the comparison of the magnitude of other key and associative events of CAR-mediated carcinogenicity cannot be further assessed due to limited mechanistic investigations in mice.

In rats the key/associative events that were investigated *in vivo* in MoA studies, i.e. CYP2B1/2 induction, increased liver weight, hepatocellular hypertrophy and cell proliferation were all significantly affected at the dose of 1800 ppm despite the short duration of exposure (7 days). Liver tumours were observed in male and female at this dose level as well as at the lower dose of 900 ppm in males. At this dose level in the MoA studies, the key parameters of CYP2B1/2 induction and cell proliferation were significantly increased in females and males, respectively supporting the conclusion that the MoA is active at this dose level although the limited exposure duration may prevent identification of additional associative effects. Altogether these data provide evidence for a dose-response relationship for the proposed MoA which is consistent with the tumorigenic dose-response.

Whilst noting that CAR-dependency for the stimulation of cell proliferation has not been established and that absence of tumour induction in mice may not be fully investigated, all in all and in line with the DS, RAC considers CAR activation to be the most plausible mechanism behind the liver tumour formation in the rat.

As to the relevance to humans of this MoA, the *in vitro* studies with human hepatocytes have shown that CAR activation is also possible in humans: epsilon-metofluthrin induced expression

[1] Recently recommended by RAC for no carcinogenic classification (RAC opinion of 11/09/2015)

of CYP2B6 mRNA (maximally 2.4 fold). However, epsilon-metofluthrin did not induce replicative DNA synthesis in human hepatocytes in two *in vitro* studies using a total of 6 human donors, in contrast to rat hepatocytes where epsilon-metofluthrin transiently but statistically significantly increased cell proliferation (around 50%). PB, the positive control in the study with human hepatocytes, also induced CYP2B6 mRNA expression (maximally 16 fold), but did not induce cell proliferation statistically different to controls. In rat hepatocytes on the other hand, and in rats *in vivo*, PB induced replicative DNA synthesis, albeit moderately (around 20% and 3.5 fold, respectively). Absence of induction of replicative DNA synthesis has also been confirmed *in vivo* in chimeric mice with humanised hepatocytes. Considering that liver tumours are not observed in mice, as well as uncertainties raised by this specific experimental model (immaturity of human cell transfected, specificities associated with this strain), the interpretation of this result is limited. However, it doesadd some weight to the *in vitro* data regarding absence of induction of replicative DNA synthesis in human hepatocytes.

RAC acknowledges the argumentation of the DS in discussing the concordance between rat and human evidence for the CAR MoA. RAC further acknowledges that similar to PB, for epsilon-metofluthrin the prerequisite for tumour formation, i.e. DNA replication, does not seem to occur in human hepatocytes following induction of human CAR, in contrast to rats. Due to this qualitative difference, the liver tumours as a result of CAR-activation by epsilon-metofluthrin are considered to be of little relevance to humans.

This is in line with a recent review on the human relevance of CAR-mediated liver toxicity, for which PB is the example substance (Elcombe *et al.,* 2014). Hence, RAC agrees with the conclusion of the DS that epsilon-metofluthrin **should not be classified for carcinogenicity**.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Summary

According to the Dossier Submitter, there are no data from studies in humans, and no evidence of developmental toxicity or an adverse effect on reproductive performance in relevant animal fertility studies. Therefore, epsilon-metofluthrin does not meet the criteria for classification.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

Comparison with the criteria

Fertility and sexual function

1. In a two-generation reproduction toxicity study (Hoberman, 2005; A6.8.2(03) Doc IIIA), groups of 30 male and 30 female CD rats were treated with epsilon-metofluthrin in the diet at 0, 50, 200, 1000 & 1800 ppm (equal in the F0 generation to 0, 3.5, 15, 69 & 126 mg/kg bw/day in males; 0, 3.9, 16, 77 & 140 in females during premating period; 0, 7.6, 29, 146 & 243 mg/kg bw/day in females on lactation days 0 – 14). Clinical signs of toxicity were confined to a statistically significantly increased incidence of tremor and twitching observed in lactating females at the highest dose of 1800 ppm in F0 animals (tremor 23/30 animals; twitch 22/30

animals); and at 1000 and 1800 ppm in F1 animals (tremor 14/30 and 30/30 animals, respectively; twitch 13/30 and 30/30 animals, respectively). At 1000 and 1800 ppm, a statistically significant increase in liver weight was reported in males of the F0 generation (absolute, 16% and 18%, respectively; relative, 16% and 22%, respectively) and the F1 generation (absolute, 18% and 12%, respectively). In females, relative liver weight was statistically significantly increased at 1800 ppm in the F0 generation (6%); while absolute liver weight was increased at 1000 and 1800 ppm in the F1 generation (14% and 18%, respectively). These liver weight changes were also associated with increased incidences of hepatocellular hypertrophy.

There were no clearly treatment-related changes on body weight or food consumption.

No treatment-related effects on sperm parameters, mating performance, fertility or parturition were observed in this study. Statistically significant decreases in pup weights were reported in the 1800 ppm group from around day 21 post-partum in F1 pups (equivalent dose level 140 mg/kg bw/d) and from day 10 post-partum in F2 pups (equivalent dose level 184 mg/kg bw/d). By day 28, both F1 and F2 pup weights were decreased by approximately 15% when compared to control animals. It is thought that these decreases in pup weight, observed during the later stages of weaning were related to palatability during the change to an adult diet and not a specific developmental effect.

2. In a non-GLP range-finding reproductive toxicity study (Hara, 2002b; A6.8.2(01) Doc IIIA), groups of 20 male and 20 female Sprague-Dawley rats were treated by gavage with epsilon-metofluthrin for 2 weeks pre-mating until pregnancy day 7 (females), or until female sacrifice (males) at a dose level of 0, 5, 10 or 20 mg/kg bw/day (males) and of 0, 10, 20 or 40 mg/kg bw/day (females).

Maternal toxicity was seen at the top dose level as maternal tremor and death. No other treatment related effects, including fertility parameters, were observed.

There is no information available on effect of epsilon-metofluthrin on fertility in humans.

Developmental toxicity

The potential for epsilon-metofluthrin to cause developmental toxicity has been investigated in standard studies in rats and rabbits.

Summary table of relevant reproductive toxicity studies

Method	Results/Remarks	Reference
EC B 31 Gavage Rat, Sprague Dawley, 24 females per dose group Days 6-19 of gestation Dose levels: 0, 5, 15, 30 mg/kg bw/day in corn oil GLP	Maternal toxicity : post-dosing tremor at the top dose Developmental toxicity: No skeletal or soft tissue malformations or variations	A6.8.1(01) Doc IIIA Hara, 2002a
EC B31 Gavage Rabbit, New Zealand White, 24 females per dose group, Days 6-27 of gestation Dose levels: 0, 25, 125, 250 mg/kg bw/day in corn oil GLP	Maternal toxicity : Mortality at 125 mg/kg bw/day and above Developmental toxicity: No skeletal or soft tissue malformations or variations	A6.8.1(02) Doc IIIA Horie, 2002

Non Standard method Gavage Rat, Sprague Dawley Day 6 of gestation to day 20 post partum Doze levels: 0, 5, 15, 30 mg/kg bw/day (in corn oil) GLP Non-guideline, but considered to be acceptable during the biocides review	Maternal tremor and death at top dose level. No other treatment-related effects	A6.8.2(02) Doc IIIA Hara, 2002c

There was no evidence of developmental toxicity in any of these studies. There is no information available on effect of epsilon-metofluthrin on fertility or development in humans.
Taking into account that no effect was observed on fertility and development of animals in the acceptable studies, RAC is of the opinion that epsilon-metofluthrin **does not warrant classification for reproductive toxicity.**

RAC evaluation of aspiration toxicity

Summary of the Dossier Submitter's proposal

No classification was discussed for aspiration toxicity.

Comments received during public consultation

No specific comments were received.

Assessment and comparison with the classification criteria

No human data are reported to indicate any potential for aspiration hazard and the substance is not a hydrocarbon. Epsilon-metofluthrin **does not fulfil the criteria to be considered an aspiration hazard**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Degradation

Hydrolysis

Metofluthrin is considered stable under acidic and neutral pH, although under alkaline conditions (pH 9) hydrolysis is slight.

The available hydrolysis study (Ponte, 2004a) carried out according to OECD TG 111 and GLP used Z and E radio-labelled [^{14}C] epsilon-metofluthrin isomers. Initial tests have shown that there

was no significant difference in the behaviour of the Z and E isomers, therefore the methoxymethylbenzyl-α-^{14}C (Alc-*Z*) and carbonyl-^{14}C labels (Acid-*Z*) were used for testing.
In a preliminary test, hydrolysis was assessed at pH 4 and 7; the Alc-*Z* isomer used was stable. The definitive study was performed at pH 9 (up to 33 days at 25°C and 8 days at 40°C), using both Alc-*Z* and Acid-*Z* labels. The DT_{50} of Alc-*Z* and Acid-*Z* determined at 25°C at pH 9 were reported as 30.7 and 33.5 days, respectively. At an elevated temperature (40°C) these DT_{50} were reduced to 4.9 and 5.1 days, respectively. These were extrapolated by calculation to provide the following DT_{50} at 12°C:

Alc-*Z*: 46.0 to 86.9 days
Acid-*Z*: 47.9 to 94.8 days

Photolysis

An aqueous photolysis study (Ponte, 2004b) was carried out in accordance with the method indicated by Council Directive 91/414/EEC, Annex II, 2.9.2 and 2.9.3 as amended by Commission Directive 95/36/EEC and The Society of Environmental Toxicology and Chemistry (SETAC).
The GLP study was carried out using a Xenon lamp at pH 4 and 20°C for 7 days. Acid-*Z* and Acid-*E* were shown to degrade rapidly when exposed to artificial light in sterile pH 4 buffer solution, declining to < 23% of the applied dose at the end of the irradiation period.
Acid-*Z* and Acid-*E* degraded rapidly within the aqueous compartment to form 3 potential major degradation products MFOA (alcohol), diol-1264 and $^{14}CO_2$ over a 7 d period.
The photolytic DT_{50} of Acid-*Z* and Acid-*E* was determined to be 2.4÷2.6 and 2.2÷2.5 days, respectively.

Biodegradation

Ready biodegradability was tested in one study (Matsumoto, 2000) following GLP and OECD TG 301C (Modified MITI [Ministry of International Trade and Industry, Japan] Test), using the test substance epsilon-metofluthrin (S-1264, purity 99.2%).
Epsilon-metofluthrin degradation based on Theoretical Oxygen Demand (TOD) was found to be very low (only 2%) after 28 d exposure. This was further supported by the mean residual rates of epsilon-metofluthrin calculated from direct determination using gas chromatography (GC), which were 96% and 95% for the test substance and abiotic control (aniline), respectively. Results suggest epsilon-metofluthrin (S-1264) is not readily biodegradable under the conditions of the test.

A soil simulation study reported DT_{50} values of 2.3÷3.5 days at 25°C equating to a mean DT_{50} of 7.4 days at 12°C. During the study, the substance rapidly degraded to primary metabolites in soil which mineralised to CO_2 with varying half-lives. The variation in mineralisation rates does not provide good evidence that epsilon-metofluthrin has an ultimate degradation half-life < 16 days (i.e. 70% degradation in 28 days).

Consequently, epsilon-metofluthrin is considered not rapidly degradable for the purpose of classification and labelling.

Aquatic bioaccumulation

The measured log K_{ow} for both the Z and E isomers of epsilon-metofluthrin is 5.0. This value was used in the QSAR equation developed by Veith *et al*. (1979) (EC, 2003):

$$\log BCF_{FISH} = 0.85 * \log K_{ow} - 0.70$$

Using this equation, logBCF is 3.55, and the predicted BCF is 3548 L/kg.
The Ds noted that the QSAR is applicable to epsilon-metofluthrin as the equation is valid for substances with log K_{ow} between 2 and 6.

The DS has analysed an epsilon-metofluthrin bioconcentration study in fish (Yakata, 2002). In this study (GLP compliant, OECD TG 305C bioconcentration test), common carp (*Cyprinus carpio*) were exposed for 60 days to two target concentrations 0.50 and 0.05 µg/L. In line with the Guideline applied at that time, no depuration phase was carried out.
Experimental steady-state BCF values were determined to be 110 L/kg (for 0,50 µg/L concentration) and 120 L/kg (for 0.05 µg/L concentration). The fish lipid content was 3.61% at the start of the study and 3.42% at the end. The arithmetic mean of these two values was used to calculate the lipid normalised (5%) BCF values. These were 156 and 171 for 0.5 µg/L and for 0.05 µg/L exposure levels, respectively. As no depuration phase was run, a DT_{50} in fish tissue for the active substance could not be established.
The isomeric purity was not known for the test substance used in the study. It is noted that the $logK_{ow}$ for both the E and the Z isomers is the same value.

The DS concluded that the measured BCF data are preferred to the predicted value, the difference presumably attributable to metabolism. The BCF of 156-171 L/kg suggests epsilon-metofluthrin is not significantly bioaccumulative according to CLP (BCF < 500).

Aquatic toxicity

Several acute aquatic toxicity data are available, all performed according to GLP and standard guidelines.

The ecotoxicity tests were conducted on a test substance with >80% Z isomer. The toxicity of other isomers was not evaluated. No aquatic toxicity data are available for the metabolites of epsilon-metofluthrin.

Valid ecotoxicological data are available for all three trophic levels. The lowest reliable ecotoxicity results in the CLH report were as follows (the key data are highlighted in bold).

Method	**Test organism**	**Test system**	**Endpoint mg/L**	**Remarks**	**Reference**
OECD TG 203, GLP 95.4%	Rainbow Trout *(Oncorhynchus mykiss)*	96h Flowthrough	**LC_{50} 0.0012** (mm)		Lima, 2004
OECD TG 203, GLP 94.9%	Common Carp *(Cyprinus carpio)*	96h Flowthrough	LC_{50} 0.00306 (mm)	tested material: mixture of Alc-Z and Alc-E (88:12) diluted with non-labelled epsilon-metofluthrin	Gries, 2002
OECD TG 202, GLP 95.4%	*Daphnia magna*	48h Flowthrough	**EC_{50} 0.0047** (mm)		Putt, 2004
OECD TG 201, GLP 95.4%	*Pseudokirchneriella subcapitata*	72h Static	E_rC_{50} 0.37 **NOE_rC 0.11**		Hoberg, 2004
mm refers to mean measured					

From the available acute aquatic toxicity data, fish are the most sensitive trophic group with $L(E)C_{50}$ values in the range 0.001 to 0.01 mg/L. In particular, the most sensitive species tested is rainbow trout (*Oncorhynchus mykiss*). Fish were exposed to the test substance in a flowthrough test system for 96h. The LC_{50}=0.0012 mg/L is based on mean measured concentrations.

The only chronic data available is the 72h NOE_rC from the algal test, which was <1 mg/L.

The DS carried out the environmental hazard assessment and proposes to classify the substance as Aquatic Acute 1 (M=100) and Aquatic Chronic 1 (M=100).

Comments received during public consultation

No comments were submitted during public consultation on the proposed environmental classification.

Assessment and comparison with the classification criteria

Degradation

Taking into account all the data provided by the DS on degradation of epsilon-metofluthrin, RAC agrees with the DS proposal to consider epsilon-metofluthrin as not readily biodegradable and not rapidly degradable.

Bioaccumulation

RAC agrees with the DS proposal that the measured BCF data are preferred to the predicted value. The measured BCF value is below 500, therefore epsilon-metofluthrin is not significantly bioaccumulative according to CLP criteria.

Aquatic toxicity

Acute aquatic hazard

Acute toxicity data are available for all three trophic levels. Fish and invertebrates are the most sensitive trophic groups with L(E)C_{50} values in the range 0.001 to 0.01 mg/L. The lowest reliable value is a 96h LC_{50}=0.0012 mg/L (mean measured concentration) for the fish species: rainbow trout (*Oncorhynchus mykiss*).

Based on acute aquatic toxicity data with short-term LC_{50} values below 1 mg/L, RAC is of the opinion that epsilon-metofluthrin should be classified as **Aquatic Acute 1; H400**. An acute **M-factor of 100** is applicable based on a value of LC_{50} being in a range 0.001 < L(E)C_{50} ≤ 0.01 mg/L.

Chronic aquatic hazard

Adequate chronic toxicity data for all trophic levels are not available. The only chronic data point is the 72h NOE_rC of 0.11 mg/L in the algal test, which is the least sensitive taxanomic group. Due to the higher sensitivity of fish or invertebrates demonstrated in the acute tests, and the lack of a complete chronic dataset, it can be concluded that there are insufficient chronic data for classification. The surrogate approach using both the available chronic and the acute data should be used.

The only chronic data (NOE_rC 0.11) is between 0.1 and 1 mg/L hence classification based on table 4.1.0 (b)(i) of Regulation 1272/2008 is Chronic 2.

For substances for which adequate chronic toxicity data are not available, the flowing criteria for Category Chronic 1 specified in table 4.1.0 (b)(iii) of Regulation 1272/2008 should be met for the other taxa:

- 96h LC_{50} (for fish) ≤ 1 mg/L and/or
- 48h EC_{50} (for crustacea) ≤ 1 mg/L and/or

- 72 or 96h E_rC_{50} (for algae or other aquatic plants) ≤ 1 mg/L.
- and the substance is not rapidly degradable and/or the experimentally determined BCF ≥ 500 (or, if absent, the log Kow ≥ 4).

Since the 96h LC_{50} for fish, 48h EC_{50} for crustacean (daphnia) are below 1 mg/L and epsilon-metofluthrin is not rapidly degradable, the substance meet the above classification criteria.

An M-factor of 100 is proposed based on the acute aquatic toxicity between 0.001 < $L(E)C_{50}$ ≤ 0.01 mg/L.

Epsilon-metofluthrin should be classified according to the most stringent outcome of the surrogate approach.

Taking this into account, RAC is of the opinion that epsilon-metofluthrin warrants classification as **Aquatic Chronic 1; H410 with an M-factor of 100.**

Additional references

Yamada, (2015). The effect of epsilon-metofluthrin and momfluorothrin on cell proliferation of human hepatocytes in chimeric mice, Study No. S1873

ANNEXES:

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

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

Annex 3 Records of the targeted public consultation on the carcinogenicity of epsilon-metofluthrin