Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

(2RS)-2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-1-(1H-1,2,4-triazol-1-yl)propan-2-ol; mefentrifluconazole

EC Number: -
CAS Number: 1417782-03-6

CLH-O-0000001412-86-199/F

Adopted

9 March 2018

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CLH-O-0000001412-86-199/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: **(2RS)-2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-1-(1H-1,2,4-triazol-1-yl)propan-2-ol; mefentrifluconazole**

EC Number: **-**

CAS Number: **1417782-03-6**

The proposal was submitted by the **United Kingdom** and received by RAC on **24 April 2017.**

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

PROCESS FOR ADOPTION OF THE OPINION

The United Kingdom has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **30 May 2017**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **14 July 2017**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Radu Branisteanu**

Co-Rapporteur, appointed by RAC: **Anja Menard Srpčič**

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 **9 March 2018** by **consensus**.

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 and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	(2RS)-2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-1-(1H-1,2,4-triazol-1-yl)propan-2-ol; mefentrifluconazole	-	1417782-03-6	Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H317 H400 H410	GHS07 GHS09 Wng	H317 H410		M = 1 M = 1	
RAC opinion	TBD	(2RS)-2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-1-(1H-1,2,4-triazol-1-yl)propan-2-ol; mefentrifluconazole	-	1417782-03-6	Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H317 H400 H410	GHS07 GHS09 Wng	H317 H410		M = 1 M = 1	
Resulting Annex VI entry if agreed by COM	TBD	(2RS)-2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-1-(1H-1,2,4-triazol-1-yl)propan-2-ol; mefentrifluconazole	-	1417782-03-6	Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H317 H400 H410	GHS07 GHS09 Wng	H317 H410		M = 1 M = 1	

GROUNDS FOR ADOPTION OF THE OPINION

RAC General comment

Mefentrifluconazole is a new fungicidal active substance for use in plant protection products that is not currently listed in Annex VI of the CLP Regulation (EC) 1272/2008.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

At 20°C and 101.3 Pa, mefentrifluconazole is a white crystalline powder. The melting point, presented as an onset value, is 125/126 °C and the decomposition onset temperature is approximately 300 °C. According to the Dossier Submitter (DS), the following physical hazard endpoints are not fulfilled: Flammable gases (including chemically unstable gases), Oxidising gases, Gases under pressure, Flammable liquids, Pyrophoric liquids and Oxidising liquids. Also, the endpoint Organic peroxides is not applicable since mefentrifluconazole does not contain peroxo-moieties. In addition, there were no data addressing the Corrosive to metals endpoint.

Explosive properties

Mefentrifluconazole was tested for explosive properties. The differential scanning calorimetry (DSC) showed two reactions:

- 1st reaction: onset 110 °C, energy intake 110 J/g (endothermic);
- 2nd reaction: onset 340°C, energy releases 580 J/g (exothermic).

Using conventional methods, the friction test was negative as well as the shock test. The thermal sensitivity test (Koenen test) showed no explosion in a test series.

Based on this battery of tests, mefentrifluconazole was not found to be sensitive to the effects of flame, shock or friction; the DS concluded that no further testing is necessary and that the substance does not meet the criteria for classification.

Flammable solids

Mefentrifluconazole was tested for flammable properties. During the test, no ignition by flame was observed and the substance melted.

Self-reactive substances

The DS stated that no test was performed since the melting point of mefentrifluconazole was below 160 °C.

Pyrophoric solids

The DS did not provide test data since the practical experience in handling and use showed that mefentrifluconazole is stable in contact with air at room temperature for prolonged periods of time.

Self-heating substances

No test had been performed since the melting point of mefentrifluconazole is below 160 °C.

Substances which in contact with water emit flammable gases

Mefentrifluconazole does not contain metals or metalloids and the experience with handling and use indicate that it does not emit flammable gases in contact with water.

Oxidising solids

Mefentrifluconazole was subjected to a burning test according to the method for Oxidising properties (solids). The results indicated that a mixture of mefentrifluconazole with cellulose had a lower maximum burning rate (1.07 mm/s) than a mixture of barium nitrate with cellulose (1.27 mm/s).

Comments received during public consultation

One Member State Competent Authority (MS) commented on the classification for explosive properties. The appropriateness of the test battery for classification was contested, namely that the EU A.14 method for explosive properties does not entirely correspond to the CLP requirements. In order to validate the proposed classification, at least a "Time/pressure test" should have been performed according to the specifications of test methods under the United Nation scheme (see below).

Assessment and comparison with the classification criteria

Explosive properties

The CLP Regulation (Section 2.1) and the CLP Guidance state that the classification for explosive properties is almost entirely adopted based on Part I of the UN Recommendations on the Transport of Dangerous Goods (UN RTDG; Manual of Tests and Criteria), which are appropriate for transport and also storage of packaged explosives. The classification of substances, mixtures and articles in the class of explosives and further allocation to a division is a very complex procedure (see also the section supplemental information, in depth analysis by RAC).

Mefentrifluconazole was investigated under a test battery which cannot be directly related to the CLP regulatory text. However, the results proved negative in three relevant key areas: behaviour to heat, shock and friction.

In conclusion, RAC considers that there are sufficient data to conclude that mefentrifluconazole should not be classified for Explosive properties under the CLP Regulation. Consequently, although through a different argumentation, the DS' proposal of **no classification** is supported.

Flammable solids

Mefentrifluconazole is a powdered material and was firstly tested in a screening procedure as specified in section 2.7.4.2 of the CLP Regulation (method EU A.10). In the test mefentrifluconazole failed to ignite by flame and melted. Consequently, a further burning rate test was not necessary. According to the decision logic for flammable solids presented in Figure 2.4 of the CLP criteria, if a screening test is negative than the substance should not be classified. Therefore, RAC agrees with the DS and supports the proposal for **no classification** as a Flammable solid.

Self-reactive substances

The CLP criteria do not contain a specific mention for waiving the evaluation as given in the CLH report. The classification in the self-reactive hazard class is not straightforward and the information provided in the physical hazard section is not sufficient for a conclusion to be drawn.

Although the reactivity profile appears low, the relationship with the other endpoints is not sufficient for a proper assessment of this property. Therefore, RAC considers that mefentrifluconazole **cannot be assessed for the self-reactive endpoint due to lack of data.**

Pyrophoric solids

According to the additional classification considerations in the CLP Regulation (section 2.10.4), the classification procedure for pyrophoric solids need not be applied when experience in manufacture or handling shows that the substance or mixture does not ignite spontaneously on coming into contact with air at normal temperatures (i.e. the substance or mixture is known to be stable at room temperature for prolonged periods of time (days)). Therefore, RAC agrees with the DS' proposal for ***no classification*** for this property.

Self-heating substances

According to the CLP Regulation, section 2.11.4.2 screening procedures and waiving of testing, the onset melting temperature is 125/126 °C for mefentrifluconazole. Even though a temperature at which the substance is completely molten is not specifically given, the melting behaviour was characterised and the cut-off value for classification is considerably higher. Therefore, RAC agrees with the DS' proposal for ***no classification***.

Substances which in contact with water emit flammable gases

According to the CLP Regulation section 2.12.4.1, the classification procedure for this class need not be applied if a) the chemical structure of the substance or mixture does not contain metals or metalloids; or b) experience in handling and use shows that the substance or mixture does not react with water, e.g. the substance is manufactured with water or washed with water. Consequently the test can be waived and RAC agrees with the DS that mefentrifluconazole should ***not be classified*** as a substance which in contact with water emits flammable gases.

Oxidising solids

According to the CLP Regulation, section 2.14.4.1.1, mefentrifluconazole does not fulfil the criteria for classification with method EU A.17. However, the method was used to deduce the oxidising properties of a solid substance under the Dangerous Substance Directive (DSD, 67/548/EEC) and Dangerous Preparations Directive (DPD, 1999/45/EC). The CLP Regulation recognises as valid for classification of solid substances for oxidising properties the test methods compiled in UN RTDG, namely UN O.1 Test for oxidising solids and UN O.3 Gravimetric test for oxidising solids. However, the substance was tested under a method previously used in classification and proved negative. In addition, similar triazole compounds already in use are not known to have oxidising properties (see also the section supplemental information, in depth analysis by RAC).

Consequently, RAC agrees with the DS that mefentrifluconazole should ***not be classified*** for Oxidising properties under the CLP Regulation.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

A total of three guideline-compliant studies, one for each acute toxicity endpoint, were included in the CLH report:

Oral route

Mefentrifluconazole of 98.8% purity was administrated by gavage to three fasted Wistar female rats. The test substance was prepared in corn oil at a concentration of 2000 mg/kg bw. Following the treatment, no deaths were registered and the clinical signs were indicative of general toxicity: cowering position, impaired general state and piloerection in the interval 2-5 hours after administration. These symptoms had resolved within a few hours and at gross necropsy, no treatment-related signs were found. Moreover, the mean body weight increased within the normal range during the study period. The result was further confirmed in three additional animals treated with the same dose. The study is GLP and OECD TG 423 compliant.

Additional information – the dose range-finding studies on rabbits

In addition to the rat study presented above, there are three dose range-finding studies on rabbits. These experiments were performed in preparation for a prenatal developmental toxicity study and the detailed description is given in the section dedicated to reproductive toxicity – developmental toxicity. The studies concluded that the dose of 50 mg/kg bw/day is potentially lethal to rabbits.

Dermal route

Mefentrifluconazole (98.8% purity) prepared as a 5000 mg/kg solution in corn oil, was applied for 24h under semi-occlusive dressing to male and female Wistar rats (5/sex). The application site was then washed with water and observed for 30-60 min, then weekly and on the last day of the test period. During the period of observation there were no signs of systemic toxicity. At gross necropsy, no treatment-related findings were found. Also, the mean body weight increased within the normal range during the study period. The study was GLP and OECD TG 402 compliant.

Inhalation route

Five male and five female Wistar rats were exposed head/nose to aerosolised mefentrifluconazole (purity 98.8%) at a concentration of 5.3 mg/L for a period of 4 hours. None of the animals died and the following symptoms were recorded: laboured breathing, abdominal respiration, respiratory sounds, encrusted eyes, red and colourless discharge and/or red crusts of the nose, poor general state, hunched posture, hyper-excitability, no defecation, piloerection and substance-contaminated fur. These clinical signs were recorded from 2 hours to 11 days after exposure but none were observed from day 12 onwards. The DS noted that the relative humidity (19%) was less than that recommended in the test guideline (30-70%), because of the need to use compressed air for dust generation; however, the study authors did not consider that this would have influenced the test results because of the relatively short exposure time.

Comments received during public consultation

No comments were provided during the public consultation.

Assessment and comparison with the classification criteria

Oral route

In rats, the oral LD_{50} for mefentrifluconazole was higher than 2000 mg/kg bw. Since classification for Acute Tox. 4 (oral) is applicable where 300 < LD_{50} ≤ 2000 mg/kg bw, no classification is warranted based on the rat data.

In rabbits, at doses of 50 mg/kg bw/day and above, the animals showed marked gastrointestinal tract (GIT) disorders: food consumption reduced to almost zero levels and there were no faeces. These manifestations proved sufficiently severe to be incompatible with life and the animals had to be sacrificed. Therefore the dose of 50 mg/kg bw/day was considered lethal. While the reason for the cessation or drastic reduction of food intake after administration of mefentrifluconazole is not entirely clear, the necropsy evidence showed local irritation and GIT segments empty of contents. These findings suggested that the rabbits did not tolerate the treatment, the condition worsened due to continued gavage and the animals became incapable of feeding themselves. This condition is very serious in rabbits due to their particular digestive physiology: the peristaltic action is lower than in rats and dogs and their digestion relies on cecotrophy. The rabbits are hindgut fermenters, the continued faecal output is vital, since rabbits depend on vitamin supply via oral uptake of their own cecotrophes (vitamin-enriched faeces via gut microflora fermentation). In addition, their defence mechanism against GIT hazards is weakened by the fact that, by contrast to e.g. dogs, they cannot vomit. These particularities make this species more vulnerable to substances that are not readily absorbed from the GIT.

These drastic effects were not observed at similar doses in the other species (rat) used in the assessment of mefentrifluconazole. Therefore, it is considered that these manifestations are specific to rabbits due to their digestive physiology which is not relevant to humans. Consequently, RAC considers that the findings in these rabbit dose-range findings (DRFs) studies are not appropriate to be considered in the evaluation of acute toxicity. Therefore, ***no classification*** is proposed based on the study on rats.

Dermal route

Classification for Acute Tox. 4 (dermal) is applicable where 1000 < LD_{50} ≤ 2000 mg/kg bw. The dermal LD_{50} for mefentrifluconazole was > 5000 mg/kg bw, therefore ***no classification*** is warranted.

Inhalation route

Classification for Acute Tox. 4 (inhalation of dusts and mists) is applicable where 1 < 4h-LC_{50} ≤ 5 mg/L. Mefentrifluconazole was tested at the concentration of 5.3 mg/L and no deaths occurred. Moreover, the noted clinical signs disappeared within the recommended 14 days of observation. These effects were not seen as significant and the option of ***no classification*** is considered appropriate.

Conclusion

Overall, RAC agrees with the argumentation presented by the DS and supports the proposal of **no classification** for acute toxicity via all routes of exposure.

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

Summary of the Dossier Submitter's proposal

The assessment of the STOT SE hazard class uses the information from the standard acute toxicity tests previously presented. In addition, an OECD TG 424 *Neurotoxicity Study in Rodents* was taken into account; the study is GLP compliant.

Neurotoxicity study on rats

Mefentrifluconazole of 98.6% purity, prepared in 1% carboxymethyl-cellulose, was administrated by gavage to two groups of rats (10/sex). The doses were 0, 200, 600 and 2000 mg/kg bw/day and the observation period was 14 days.

No deaths and/or clinical signs of toxicity were recorded in any group. The body weights of the treated animals were not significantly different from the controls. Mean body weight gains at the high dose were lower in males (by 29%, statistically significant) and females (by 20%, not statistically significant) between study days 0 to 7. However, by day 14 all the animals had recovered and no differences were recorded at the end of the study.

The functional observation battery (FOB) was performed prior to treatment and on study days 0, 7 and 14. At the mid and low doses no changes were observed. At the high dose of 2000 mg/kg bw/day unsteady gait was observed in 5/10 males and 3/10 females on day 0. This finding was not observed on days 7 and 14 and was explained by the DS as being related to the bolus dosing of a high dose. In summary, the sensorimotor parameters were not affected by the treatment.

Other findings on day 0 were decreased limb strength of the forelimbs and an increased foot-splay in the high dose group. The effects were not evident on days 1 and 14. Therefore, the DS concluded that the increased foot-splay did not arise from structural neuronal damage but was instead a manifestation of general toxicity. An increase in the landing foot-splay distance was also noted at the mid dose but the value was consistent within the historical control data (HCD) range.

The motor activity was measured on the same days as the FOB and the sole disturbance was recorded on day 0 in the mid and high dose groups.

At necropsy no treatment-related gross or histological findings were found.

Overall, the acute neurotoxicity study on rats did not provide evidence of neuro-behavioural toxicity. The transitory findings may be attributed to systemic toxicity.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

Mefentrifluconazole administrated orally at 2000 mg/kg bw/day induced transitory general toxicity. However, the observed clinical signs gave no indication of specific target organ toxicity and the gross pathology did not reveal any adverse findings. Also, mefentrifluconazole administrated dermally at a dose of 5000 mg/kg bw/day did not induce any treatment-related findings at gross necropsy. Overall, no specific target-organ toxicity was identified at doses equal to the top of the guidance value range listed in the CLP Regulation (Annex I: 3.8.2.1.9.3, Table

3.8.2 *Guidance value ranges for single-dose exposures)*. Accordingly, mefentrifluconazole does not meet the criteria for classification for STOT SE Categories 1 or 2 under the CLP Regulation.

STOT SE Category 3 is assigned for respiratory tract irritation (RTI) and/or narcotic effects. Mefentrifluconazole administrated by inhalation at a concentration of 5.3 mg/L for a period of 4 hours induced clinical general signs of toxicity for a limited time. The respiratory effects were severe but transitory, thus raising the option of classification. Indeed, the symptoms were associated with exposure and lasted for 11 days out of a total observation period of 14 days. While some findings can be associated with general toxic effects, the laboured breathing and respiratory sounds suggest respiratory irritation. The CLP Regulation sets a series of indications for respiratory irritation (see Annex I, section 3.8.2.2.1 *Criteria for respiratory tract irritation*). While in humans the descriptions are clear, in animals the text specifies that:

(d) 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 and 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 weight of evidence evaluation.

In the present case the necropsy did not reveal any macroscopic findings and no histopathological data is given. While the transitory effect is the very characteristic of STOT SE 3, the lack of data in support of the respiratory symptoms prevents an unequivocal association with respiratory tract irritation specifically caused by intrinsic properties of mefentrifluconazole. Therefore, RAC considers that the described symptoms are indicative but not sufficient to warrant a classification as STOT SE 3 for respiratory tract irritation.

With respect to the narcotic effects, there were no indications of narcosis or impaired consciousness in any of these studies. Therefore, the findings from the respiratory exposure do not support classification in any category (1, 2 or 3) according to the CLP Regulation (Annex I: 3.8.2.1.1, Table 3.8.1 *Categories for specific target organ toxicity-single exposure)*.

In conclusion, RAC agrees with the DS proposal for **no classification** for specific target organ toxicity – single exposure (STOT SE).

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

To evaluate the skin corrosion/irritation potential of mefentrifluconazole the DS summarised one *in vitro* and one *in vivo* study in the CLH report.

The EpiDerm™ skin model was used for the *in vitro* test. The Human reconstituted epidermis model was exposed to 25 µL bulk volume (approximate 11 mg) of 97.7% purity mefentrifluconazole for:

- 3 minutes and one hour in the corrosion test;
- one hour with 42 hours pot-incubation in the irritation test.

The measured tissue viability values were comparable to the negative control (100-102% of the negative control values) indicating that the test substance is not a skin corrosive/irritant substance.

The *in vivo* study followed the protocol OECD TG 404 was GLP compliant. Mefentrifluconazole (0.5 g of 98.8% purity) was applied to intact skin of 3 New Zealand White (NZW) rabbit females in a step-wise procedure. The exposure lasted 4 hours under semi-occlusive dressing. The scores

expressed as the average over 24, 48 and 72 hours were zero for both erythema and oedema in each animal.

Comments received during public consultation

No comments were received during the public consultation.

Assessment and comparison with the classification criteria

Under the CLP Regulation, a corrosive substance is defined as "*a substance that produces destruction of skin tissue, namely, visible necrosis through the epidermis and into the dermis".* The Reconstructed Human Epidermis Test Method using EpiDerm™ skin model is a suitable *in vitro* method for the hazard identification of corrosive and irritant chemicals under CLP. The working protocols of this commercially available assay system fully comply with OECD TG 431 (corrosion) and OECD TG 439 (irritation) test guidelines. The results for mefentrifluconazole indicate that the test substance is not a skin corrosive substance. This conclusion is further supported by the *in vivo* test.

Based on the *in vivo* test none of these criteria are fulfilled. Moreover, the *in vivo* test showed that mefentrifluconazole does not have skin irritative potential. Therefore, RAC agrees with the DS proposal for **no classification** for skin corrosion/irritation.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS provided two *in vitro* and one *in vivo* studies.

The Reconstructed human Cornea-like Epithelium (RhCE) test using commercially available EpiOcular™ Eye Irritation Test was first employed as a pre-test. The assay used two EpiOcular tissue samples where 50 µL (approx. 15 mg) of mefentrifluconazole (97.7%) minimally moistened with water was applied. The exposure time was of 90 minutes followed by a period of 18 hours of post-treatment incubation. The results indicated a tissue viability of 81% and 20% for the negative and the positive control (methyl acetate), respectively.

The second *in vitro* test was the Bovine corneal opacity and permeability (BCOP) test. Three bovine corneas were used and a volume of 750 µL watery solution of 20% mefentrifluconazole (97.7% purity) was applied. The *in vitro* irritancy score (IVIS) for the test substance was of -0.4±2.1 and the histopathology did not reveal indications of eye damage. The test is OECD TG 437 and GLP compliant.

The follow up *in vivo* test was conducted according to the OECD TG 405 Acute Eye Irritation/Corrosion test protocol. One animal was used initially establish the potential for severe lesions then another two animals were added. Bulk volume (0.1 mL, approx. 38 mg) of mefentrifluconazole (98.8%) was applied in one eye for 24 hours followed by rinsing with tap water. The results of the three animals tested are summarised in the following table:

Score*	Cornea (min.0-max.4)	Iris (min.0-max.2)	Conjunctivae (min.0-max.3)	Chemosis (min.0-max.4)
Animal #1	0	0	0.3	0
Animal #2	0	0	0.3	0
Animal #3	0	0	0.7	0

*All values are reported as the mean of the 24, 48 and 72h observations

Grade 1 conjunctival redness was noted in all three rabbits at 1 and 24 hours after treatment and persisted in one animal for up to 48 hours. Also, slight conjunctival chemosis was noted in one animal at hour 1 after application. In two rabbits a slight discharge was noted at hour 1 after application. In addition, injected sclera vessels in a defined area were noted in all animals at 1 hour and persisted in two animals for up to 24 hours.

All the reactions were reversible within 72 hours after application. Also, the additional examination with instillation of fluorescein performed at 24 and 48 hours after treatment could not detect any corneal lesion.

Comments received during public consultation

No comments were received during the public consultation.

Assessment and comparison with the classification criteria

The EpiOcular assay is an *in vitro* test system routinely employed in assessing the irritation potential of a chemical substance. In a similar way to the method using reconstructed human tissue used for testing the dermal toxicity, the RhCE method assesses with a colorimetric test the cell viability of a human cornea-like epithelium after exposure/post-incubation. At the time the CLH report was written, the EpiOcular test was not validated for regulatory purposes and the DS presented the test as being neither guideline nor GLP compliant. However, the method was in the meantime adopted (on the 9th October 2017) as OECD TG 492. Taking note of this modification, RAC agrees with the DS that there is no indication of irritation potential.

The BCOP test had an IVIS score of -4, which is below the cut-off value of 3 indicative for classification for eye irritation or serious eye damage.

The *in vivo* eye irritation/corrosion test resulted in scores well below the threshold for classification under CLP; the noted adverse effects were mild and reversible in a very short time.

In conclusion, since the substance does not have physical-chemical properties to support classification, and the animal data showed no potential for eye damage/ irritation, RAC agrees with the DS proposal for **no classification** of mefentrifluconazole for serious eye damage/eye irritation.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

Mefentrifluconazole was tested in a OECD TG 406 and GLP compliant Guinea Pig Maximisation Test (GPMT). The test and groups consisted of 10 and 5 Dunkin-Harley female guinea pigs, respectively. The intra-dermal induction used 5% w/w suspension of 98.8% purity mefentrifluconazole in paraffin oil and 5% w/w in Freund's complete adjuvant (FCA)/0.9% aqueous NaCl. The topical induction consisted of 60% w/w test substance in paraffin oil and the challenge used 50% w/w in the same vehicle.

The concentrations employed in the induction and challenge phases were determined from pre-tests. Also, the concentrations used in the topical induction and challenge were selected based on the minimal irritating concentration observed in two animals.

The response after challenge of the test and control groups are summarised in the following table:

	24h	48h	Total
Control	0/5	0/5	0/5
Test	2/10	6/10	6/10

RAC notes that the intra-dermal injection of the 5% mefentrifluconazole in the adjuvant mixture caused grade 1 and 2 irritation and necrosis. Necrosis was also observed during the topical induction phase at the sites of adjuvant administration in both the control and the test groups but not at the injection sites without adjuvant. The sites of injections with only the test substance or vehicle showed no or slight erythema and no necrosis.

During the challenge phase no skin reactions were observed in the control group. In the test group, 6 out of 10 animals exhibited grade one erythema and two animals additionally presented papules at 24 and/or 48 hours after the challenge. The challenge treatment with the vehicle alone did not cause skin reactions in the test group. The DS proposed to classify mefentrifluconazole as Skin Sens. 1 (H317) in the absence of a possibility to sub-categorise the positive effects observed.

Comments received during public consultation

During the public consultation, no specific comment was received.

Assessment and comparison with the classification criteria

The results of the GPMT show that 60% of the test animals gave a positive response and this demonstrates that mefentrifluconazole has skin sensitisation potential. RAC notes that 60 % of the test animals responded to a 5 % intradermal induction dose and thus the substance would deserve a classification in at least sub-category 1B according to the CLP Regulation (Annex I: 3.4.2.2.3.2, Table 3.4.3 corroborated with Annex I: 3.4.2.2.3.3, Table 3.4.4). However, since an intra-dermal induction concentration below 0.1 % was not tested, RAC agrees with the DS that is not possible to exclude a classification in sub-category 1A. In this situation, the guidance on the application of the CLP criteria recommends that the default position of classification in category 1 be adopted, i.e., without sub-categorisation.

Therefore, although it is recognised that mefentrifluconazole is unlikely to be a potent skin sensitiser, RAC supports the proposal of the DS to classify mefentrifluconazole as **Skin Sens. 1; H317 – May cause an allergic skin reaction.**

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

Summary of the Dossier Submitter's proposal

The STOT RE of mefentrifluconazole was investigated in 28-day and 90-day studies in rats, mice and dogs and a 12-month study in dogs. In addition, data from the chronic/carcinogenicity studies in rats and mice have been used; the study descriptions are presented in the Carcinogenicity section. Also, the dermal repeated dose toxicity was investigated in a 28-day study in rats. A short consideration of the rabbit dose range-finding studies submitted for the assessment of reproductive toxicity was also made.

The summary of the test conditions (with the exception of the chronic/carcinogenicity study and the rabbit studies) are presented in the following table:

Test method and duration	**Species strain**	**Substance purity and dose levels**	**CLP Guidance values for STOT RE 1 (cat 1) or STOT RE 2 (cat 2)** *(mg/kg bw/day)*
28-day oral (dietary) OECD TG 407	Rat, Wistar 5/sex/group	-Purity 97.7% -0, 500, 1500, 4000 ppm, equivalent to Males: 0, 47, 135, 388 mg/kg bw/day Females: 0, 47, 138, 334 mg/kg bw/day	Cat 1 = 30 Cat 2 = 300
28-day oral (dietary) OECD TG 407	Mouse, C57BL/6 Rj 5/sex/group	-Purity 95.5% -0, 30, 100, 300, 1000 ppm, equivalent to Males: 0, 4.8, 15.5, 47.9, 128 mg/kg bw/day Females: 0, 5.8, 18.5, 61.0, 145 mg/kg bw/day	Cat 1 = 30 Cat 2 = 300
28-day oral (capsule) OECD TG 407	Beagle dog (range-finding study) 3/sex/group	-Purity 98.6% -Males: Days 1-2: 300 or 1000 mg/kg bw/day Days 7-35/36: 125 or 250 mg/kg bw/day -Females: Day 1: 300 or 500 mg/kg bw/day Days 3-29/30: 125 or 250 mg/kg bw/day	Cat 1 = 30 Cat 2 = 300
90-day oral (dietary) OECD TG 408	Rat, Wistar 10/sex/group	-Purity 95.5% -0, 400, 1200, 3600 ppm, equivalent to Males: 0, 27, 76, 256 mg/kg bw/day Females: 0, 30, 91, 314 mg/kg bw/day	Cat 1 = 10 Cat 2 = 100
90-day oral (dietary) OECD TG 408	C57BL/6 Rj mouse 15/sex/dose	-Purity 98.8% -0, 10, 50, 250, 750 ppm, equivalent to Males: 0, 2, 11, 58, 174 mg/kg bw/day Females: 0, 3, 15, 67, 211 mg/kg bw/day	Cat 1 = 10 Cat 2 = 100
90-day oral capsule OECD TG 409	Beagle dog 5/sex/dose	-Purity 98.6% -0, 15, 90, 180 mg/kg bw/day (males and females)	Cat 1 = 10 Cat 2 = 100
12-month oral capsule OECD TG 452	Beagle dog 5 / sex / dose	-Purity 98.8% -0, 10, 30, 150 mg/kg bw/day (males and females)	Cat 1 = 2.5 Cat 2 = 25
28-day dermal OECD TG 410	Wistar rat 10/sex/dose	-Purity 98.6% -0, 100, 300, 1000 mg/kg bw/day 6 hours/day on 5 days/week for 4 weeks (males: 21 applications; females: 22 applications) in 0.5% carboxy-methylcellulose in drinking water	Cat 1 = 20 Cat 2 = 200

No adverse effects were reported in the dermal study regardless of the treatment dose.

In the studies employing oral administration of mefentrifluconazole for 28 days, 90 days and 12 months, the target organ was the liver. The treatment-related effects comprised increases in absolute and relative weights, clinical chemistry parameters alterations and histopathology findings.

***Doses and effects* (*, p < 0.05; **, p < 0.01)**

- The lowest doses at which effects were detected were 256 mg/kg bw/day (males, 90-days, rat) and 150 mg/kg bw/day (12 months, dog).
- In rats statistically significant findings comprised decreased body-weight gain (males: -11%**, females: -20%**), increased alkaline phosphatase (ALP) (males** and females**), increased cholesterol* plus decreased albumin** (females), increased relative liver weight (males: +11%**, females: +13%**) and increased minimal hepatocellular hypertrophy (8/10 males and 3/10 females, minimal).

- In dogs the effects comprised: increased ALP in males and females, decreased aspartate aminotransferase (AST) in males, decreased total protein (males) and albumin (males and females), decreased calcium (males and females) and creatinine (females), decreased absolute lymphocyte counts (males at 3 months), decreased absolute and relative liver weight in males (relative +33% *) and females (relative +31%). The histopathological examination of the liver revealed centrilobular or diffuse hepatocellular hypertrophy in 5/5 males and 5/5 females; also, minimal eosinophilic change (5/5 males, 5/5 females) was detected. The DS characterised these findings as indicating impairment of liver function.
- In mice, effects were detected at a much lower dose of 47.9 mg/kg bw/day (males, 28-days, dietary): increased relative liver weight (by 22%* in males and 33%* in females) and liver cell hypertrophy (5/5 for males minimal to moderate and 5/5 females minimal to slight, compared with 0/5 in each control group). The lower dose corroborated with a higher severity of the effects indicate that the mouse was the most sensitive species in this assessment.

The severity of effects

The severity of the liver effects did not markedly increase with the increase in exposure duration from 28 to 90 days, or to one year in dogs; increased liver weights with hypertrophy and eosinophilic change of hepatocytes were reported in all studies. However, the hypertrophy was reported as dose-dependent. In rats, in the 12 months study (doses up to 191/300 mg/kg bw/day in males/females) as well as in the 24 months study (doses up to 163/302 mg/kg bw/day in males/females) the same trend was noted. The increased relative weights up to 22% and minimal-to-slight hepatocellular hypertrophy (6/10 males and 5/5 females in the 12 months and 15/50 males and 7/50 females in the 24 months studies) were considered by the DS to be adaptive changes.

In mice the severity of the liver effects was higher than in rats. In the 18-month carcinogenicity study, an increase in the severity and/or incidence of hepatocellular fatty change was evident from 9.1 mg/kg bw/day in males and at 61.5 mg/kg bw/day in females. However, centrilobular eosinophilic inclusions in males and hepatocellular single-cell necrosis in females were only observed at the highest doses (36/61.5 mg/kg bw/day). When severity was analysed as a function of duration, in mice there was no clear indication that the liver toxicity became more severe with an increase in duration of exposure from 90 days to 18 months; although there were a small number of animals with a more severe grade of fatty change, all cases of necrosis were single-cell rather than multifocal and were scored as minimal. Therefore, while the mouse is the most sensitive species in terms of severity of effects versus dose, the same time pattern in toxic effects was observed in rats and dogs.

Other effects

The decreased body weight and body weight gain were also consistent findings across the three species. The effect was undoubtedly treatment-related since it appeared regardless of the mode of administration. Also, this effect was the most sensitive indicator of toxicity in rats and dogs; by contrast, in mice, the most sensitive parameter was the histopathological findings.

Other toxicity-related findings were noted but did not prove to be consistent across the studies. For example, haemoconcentration was observed in the 90-day mouse study at a dose below the guidance cut-off values for classification (58/67 mg/kg bw/day). However, the DS considered that this is not a consistent finding since there was no clear explanation (for example, no histopathological effects on the adrenal cortex, bone marrow or spleen), the effects were very slight (< 5% changes in haematology parameters) and they were not replicated in other studies, including the mouse carcinogenicity study. Also, slight changes in haematology parameters were

noted in the 12 month rat combined chronic toxicity/carcinogenicity study at the mid dose of 31/41 mg/kg bw/day but were not consistent with haemoconcentration.

Also, some histopathology changes were noted in mice following 18 months of administration in the kidney (from 9.1 mg/kg bw/day in males), adrenal glands (at 61.5 mg/kg bw/day in females) and thyroid (at 36/61.5 mg/kg bw/day in males and females). These findings generally did not represent a clear toxic effect and, in the case of the kidney and adrenal-gland changes, were not associated with degenerative processes.

Summary

As shown in the Table below, the DS reported liver effects that were observed in all studies and comprised increased liver weight, hepatocellular hypertrophy and fatty change, hepatocellular eosinophilic change, hepatocellular necrosis, oval cell proliferation and bile-duct hyperplasia. These histopathological modifications were accompanied by clinical chemistry modifications such as increases in enzymatic activity. However, the severity of these findings varied across the species, with mouse being the most sensitive species. Other toxicity-related findings were also noted but these were not consistent across the studies/species.

Based on the adjusted guidance values the DS summarised the effects observed at doses below the threshold for classification as follows:

Study	(Adjusted) guidance value for STOT RE 1 / STOT RE 2 (mg/kg bw/day)	Effects at doses below guidance cut-off values
28-d rat study	30 / 300	Category 1: Lowest dose = 47 mg/kg bw/day Category 2: No adverse effects at 47 mg/kg bw/day & 135/138 mg/kg bw/day
28-d mouse study	30 / 300	Category 1: ≤ 15.5 / 18.5 mg/kg bw/day = increased relative liver weight & hepatocellular hypertrophy (adaptive) Category 2: ≥ 47.9 / 61 mg/kg bw/day = increased relative liver weight & hepatocellular hypertrophy (adaptive) 128 / 145 mg/kg bw/day = decreased body weight, clinical chemistry changes, hepatocellular hypertrophy, necrosis (minimal/slight), oval-cell proliferation (minimal), bile duct hyperplasia (minimal)
28-d dog study	30 / 300	Category 1: Lowest dose = 125 mg/kg bw/day Category 2: 125 – 250 mg/kg bw/day = decreased body weight, increased liver weight, hepatocellular hypertrophy, eosinophilic change of hepatocytes, delayed food intake
90-day rat study	10 / 100	Category 1: Lowest dose = 27 / 30 mg/kg bw/day (no adverse effects) Category 2: No adverse effects at 76 / 91 mg/kg bw/day
90-day mouse study	10 / 100	Category 1: No adverse effects at 2 /3 mg/kg bw/day Category 2: ≥ 11 / 15 mg/kg bw/day = haemoconcentration, clinical chemistry changes 58 / 67 mg/kg bw/day = increased liver weight, liver single-cell necrosis (grade 1, 2/10 males) & cytoplasmic alteration (grade 1, 4/10 males)

Study	(Adjusted) guidance value for STOT RE 1 / STOT RE 2 (mg/kg bw/day)	Effects at doses below guidance cut-off values
90-d dog study	10 / 100	Category 1: Lowest dose = 15 mg/kg bw/day (no adverse effects) Category 2: No adverse effects at 90 mg/kg bw/day
1-year dog study	2.5 / 25	Category 1: Lowest dose = 10 mg/kg bw/day Category 2: 10 mg/kg bw/day = minimal eosinophilic change (3/5 males, 2/3 females) adaptive change; no adverse effects
2-year rat study (chronic toxicity/ carcinogenicity)	2.5 / 25 (one-year) 1.25 / 12.5 (two-year)	Category 1: Lowest dose = 4 / 6 mg/kg bw/day (no adverse effects at two years) Category 2: 31 / 41 mg/kg bw/day (12 months) = slight haematology & clinical-chemistry changes 25 / 38 mg/kg bw/day (two years) = increased relative liver weight in females
18-month mouse study (carcinogenicity)	1.7 / 17	Category 1: Lowest dose = 3.5 / 4.9 mg/kg bw/day (no adverse effects) Category 2: 9.1 / 12.6 mg/kg bw/day = increased relative liver weight with hepatocellular diffuse (microvesicular) fatty change (severity score 2.9 compared with 2.0 in controls; no change in incidence) in males; increased incidence & severity of macrovesicular fatty change in males (35/50 with mean severity 1.5 compared with 23/50 with mean severity grade 0.5 in controls).

The DS concluded that the only target organ at doses below the guidance cut-off value for category 2 was the liver. Overall, the DS concludes that the necrosis and morphological changes in the liver were not sufficiently severe or reproducible to warrant classification.

Comments received during public consultation

One comment was received by a MS during the public consultation, which suggested classification of mefentrifluconazole as STOT RE 2 based on the following pathological observations at doses below the guidance values:

- hepatocellular necrosis, oval-cell proliferation, bile-duct hyperplasia, cytoplasmic alteration, fatty change in the 28-day and 90-day studies in mice;
- increased liver weight, hepatocellular hypertrophy in 28-day mice study;
- increase in severity of hepatocellular fatty change in 18-months mice study;
- increased liver weight with hepatocellular hypertrophy and eosinophilic change of hepatocytes in one–year dog study and 28-day dog study;
- increase in ALP and ALT values 12-months rat study.

Assessment and comparison with the classification criteria

According to the CLP Regulation (Annex I, section 3.9.2.1), *Substances are classified as specific target organ toxicants following repeated exposure by the use of expert judgement, on the basis of the weight of all evidence available, including the use of recommended guidance values which*

take into account the duration of exposure and the dose/concentration which produced the effect(s).

Effects corresponding to the classification as STOT RE 1

RAC notes that the lowest test doses used in rats and dogs were higher that the threshold values for STOT RE 1; consequently, no comparison can be made. In the mouse, which is the most sensitive species, the lowest doses used in the repeated studies are below the reference values and effects in one of the two tests were noted. In the 28-day study, at the lower doses of 4.8/5.8 and 15.5/18.5 mg/kg bw/day a dose-related increase of the liver weight and liver cell hypertrophy was noted. The liver weight was statistically significant in both doses but fell within the HCD range at the lowest dose. The cellular hypertrophy was minimal at the lowest dose and minimal and moderate at the mid-dose level. These effects were not recorded in the 90 day study. Moreover, in the 18-month study in mice, the lowest doses, although higher than the threshold values, did not induce any adverse effect. The effects noted in the 28-day study were of low severity and not reproducible; consequently, RAC considers that they should not be taken into account for classification.

Overall, RAC agrees with the DS that mefentrifluconazole does not meet the criteria for classification in Category 1.

Effects corresponding to the classification in STOT RE 2

RAC notes that in rats, the repeated dose toxicity studies revealed no effects at doses below the threshold values for classification in Category 2. In the 12-month study, slight haematological and clinical chemistry modifications concurrent with liver weight increases were reported. However, the severity of these findings was low and the modifications appeared adaptive rather than toxic. In dogs, effects were reported in the dose range-finding and 1 year studies at doses below the threshold for classification in Category 2. However, the severity of these findings was low and they were considered adaptive rather than toxic effects. The severity of the eosinophilic modification was noted as minimal to slight, and therefore did not clearly indicate hepatotoxicity. In addition, all these findings were not reproduced in the 90-day study at doses below the threshold for classification.

In mice, adverse effects at doses below the threshold reference values were noted in all studies and the significance of these findings is discussed below.

<u>Adverse change in haematology and clinical chemistry in mice</u>

In the 90-day study evidence of haemoconcentration was reported only in males. The effects on haemoglobin and haematocrit consisted of low level changes (≤ 5%) and there was no histological evidence of an increased production of red blood cells. Therefore, these modifications were not a clear evidence of a toxic effect. Moreover, these effects were not reproduced in the 28-day and 18-month studies.

Modifications in cholesterol levels reported in the 28 and 90-day but not in the 18-month studies appeared treatment-related but adaptive rather than toxic effects.

In summary, RAC agrees with the DS that the haematological and clinical chemistry modifications were treatment-related but were not severe enough to be considered for classification.

<u>Multi-focal or diffuse necrosis, fibrosis or granuloma formation in mice</u>

Hepatocellular necrosis was recorded in the 28-day and 90-day studies but not in the 18-month study. In the 28-day study, the necrosis was multifocal but of minimal/slight severity. In the 90-day study the necrosis was single cell, of minimal severity and in only 20% of males, with no cases in females. Therefore, although liver necrosis *per se* is a severe effect, in this case it

appears rather isolated since it is neither dose nor time related and the incidence is low. Consequently, it does not provide a convincing evidence of severe treatment-induced effect and RAC agrees with the DS that it should not be taken into account for classification.

Fatty change in liver - mice

In the 18-month study in mice increased relative liver weights (18%) with hepatocellular diffuse (microvesicular cytoplasmic) fatty change was seen in the males from the middle dose group. This fatty change was also shown in the high dose (both males and females). Also, the diffuse fatty change was not seen in the 28 and 90-day studies.

The incidence of the fatty change did not differ from the controls but the severity was slightly increased from 2.0 in controls to 2.9 in the exposed group. In addition, in the same groups, the incidence and severity of the macrovesicular fatty change was increased but this was also the case in the concurrent control group, approaching a spontaneous incidence of 50%. Based on the high incidence in controls, corroborated by the absence of fatty liver in the shorter duration studies, the DS assumed that the finding was age-related. However, it is noted that the change was not evident at the lowest dose of the 18-month test.

The CLP Regulation exemplifies severe fatty change as a morphological change that is potentially reversible but is clear evidence of marked organ dysfunction. In the case of mefentrifluconazole, the fatty change was of only slightly increased severity when compared with controls. Moreover, the slight but well characterised fatty change cannot be seen as a clear evidence of marked organ dysfunction.

Since the severity was relatively low, marked liver dysfunction was not demonstrated and the finding was present only in the 18-month study but not in the two repeated dose toxicity tests, RAC agrees with the DS that the fatty change should not be taken into account for classification.

Oval-cell proliferation and bile duct hyperplasia

These findings were evident in the 28-day mouse study at doses below the threshold values for classification in category 2. However, in the longer duration studies the findings were not consistent and RAC agrees with the DS that they should not be taken into account for classification.

Conclusion on STOT RE

Following the tests on mefentrifluconazole in repeated and chronic/carcinogenic studies the only target organ appeared to be the liver. The toxic manifestations consisted of clinical chemistry, haematology (decreased haemoglobin and haematocrit) and histopathological findings. Overall, RAC concurs with the DS that the clinical chemistry investigations indicated that adaptive changes and some alterations to liver-cell metabolism resulted from exposure to mefentrifluconazole. This was supported by the haematology investigations, since changes in blood parameters indicated an increased synthesis of coagulation factors in the liver. RAC thus concludes that these effects in rats were treatment related but not adverse and do not support classification.

The severity of the effects differed across the species with the mouse as the most sensitive one. The lowest doses tested in rats and dogs tests were higher than the threshold values corresponding to STOT RE 1. Moreover, findings in dogs and rats met the guidance values for STOT RE 2 but RAC concludes that they are of low severity and reproducibility.

Effects in mice were evident in all the studies at doses below the CLP Regulation threshold values for both categories STOT RE 1 and 2. However when closely analysed, the findings proved either of low severity or not reproducible.

In summary, the detected effects in liver did not meet the basic requirements of a specific organ toxicity induced by repeated exposure, which are severity and consistency. Consequently, RAC

agrees with the DS and supports the proposal for **no classification** of mefentrifluconazole for STOT RE.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The mutagenicity/genotoxicity potential of mefentrifluconazole was assessed in three *in vitro* assays and one *in vivo* assay. All the tests were guideline and GLP compliant but were performed on somatic cells. The potential for germ-cell mutagenicity has been not investigated.

In vitro tests

Mefentrifluconazole was negative in two bacterial reverse mutation tests (OECD TG 471) over two concentrations ranges: 1-5000 µg/plate at 98.6% purity (Woitkowiak, 2014a) and 3.3-5000 µg/plate at 97.9% purity (Woitkowiak, 2015a) with and without metabolic activation. Both concentration ranges gave negative results. The cell cytotoxicity indicated that the substance was tested at adequate concentrations.

In mammalian cell gene mutation tests (OECD TG 476) using the Hprt and xprt genes, mefentrifluconazole was negative when tested in two concentration ranges: 3.75-60 µg/mL at 98.8% purity (Wollny, 2015a) and 3.1-62.5 µg/mL at 97.9% purity (Wollny, 2015b). The test were performed both with and without metabolic activation and gave negative results even at concentration as high as the precipitating point of mefentrifluconazole.

In mammalian cell micronucleus tests (OECD TG 487), mefentrifluconazole was negative when tested in two concentration ranges: 0.39-50 µg/mL at 98.8% purity (Landsiedel, 2014a) and 2.0-8.2 µg/mL at 97.9% purity (Sokolowski, 2015a). The test were performed on both animal (hamster lung fibroblasts) and human (lymphocytes) cells with and without metabolic activation. All the assays showed negative results.

In vivo test

In a mammalian erythrocyte micronucleus assay (OECD TG 474), mefentrifluconazole of 98.8% purity was prepared in DMSO/corn oil 2:3 and administrated by gavage to male NMRI mice. The test concentrations were of 0, 375, 750 and 1500 mg/kg bw and the number of animals was 5 per group. The treatment with mefentrifluconazole did not exhibit a biologically relevant increase in the number of micronucleated polychromatic erythrocytes. The rate of micronuclei was close to the concurrent negative control and within the range of the HCD.

All *in vitro* and *in vivo* tests were considered negative and therefore the DS did not propose a classification for germ cell mutagenicity.

Comments received during public consultation

No comments were received during the public consultation.

Assessment and comparison with the classification criteria

RAC agrees with the DS that mefentrifluconazole was clearly negative in three well-conducted *in vitro* mutation assays in bacteria or mammalian cells with and without metabolic activation. In addition, RAC agrees with the DS that mefentrifluconazole was negative a well-conducted *in vivo* micronucleus test. In conclusion, mefentrifluconazole did not show any mutagen potential in any

of the applied assays. Therefore, RAC concludes that mefentrifluconazole does not meet the criteria for classification and agree with the DS's proposal for **no classification** as a germ cell mutagen.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The carcinogenic potential of mefentrifluconazole was tested in two GLP compliant studies performed on two species.

Two-year Combined Chronic Toxicity/Carcinogenicity study in Rats

The combined chronic toxicity and carcinogenicity study was conducted according to OECD TG 453. In the chronic assay (12 months), the animals were grouped as 10/sex/dose and in the carcinogenicity phase (24 months) as 50/sex/dose. The administrated dosage was of 0, 100, 600 and 3600 ppm via the diet; the corresponding intakes were calculated as 0, 4, 25, 163 mg/kg bw/day, respectively, for males and 0, 6, 38, 302 mg/kg bw/day for females.

In the chronic assay, the DS concluded that there were no adverse effects at the low dose. At the mid-dose, changes in haematology parameters (decreased activated partial thromboplastin time in males) and clinical chemistry (increased ALP and urea in both males and females) were observed. At the high dose, decreased mean body weight (final -8.3% in males and -13.8% in females) and body weight gain (overall -12% in males and -27.1% in females) were noted. Haematology (decreased activated partial thromboplastin time in males, decreased platelet counts in males, clinical chemistry (increased ALP, cholesterol, glucose and urea and decreased total protein, albumin and creatinine in both males and females) were observed. The liver appeared to constitute the target organ with an increased relative liver weight (9% and 22% in males and females) and minimal/slight hepatocellular hypertrophy (6/10 males and 5/5 females).

The non-neoplastic effects in the carcinogenicity phase (24 months) were similar but more pronounced after 24 months of exposure. There were no apparent clinical signs of toxicity in any group and no adverse effects were recorded at the low dose. At the mid-dose, the relative liver weight was increased (16%) in females. At the high dose, decreased mean body weight (final -11.6% in males and -21.9% in females) and body weight gain (overall -15.7% in males and -35.1% in females) together with an increased relative liver weight (7 and 23% in males and females) and minimal hepatocellular hypertrophy (15/50 males and 7/50 females).

All the findings above were statistically significant at $p \leq 0.01$.

The neoplastic findings at 24 months were limited to the high dose group when compared with the controls: slight increases in the incidence of malignant lymphoma (haemolymphoreticular system) in males and uterine adenocarcinoma in females. Overall, the neoplasms findings were comparable between control and high-dose females: total number of primary neoplasms (73 vs. 65), benign neoplasms (62 vs. 53), and malignant neoplasms (11 vs. 12). The total numbers of systemic and metastasised neoplasms were comparable between control and high-dose males and females.

The DS provided an analysis of the significance of both types of tumours (malignant lymphoma of the haemolymphoreticular system and uterine adenocarcinoma in females). According to the DS, a clear dose-response relationship was not evident in the malignant-lymphoma incidences, and the high-dose group incidence (6 %) was within the wider HCD, exceeding the more recent HCD (upper range 4 %, mean 3.2 %) by just one animal. Although many of the tumours were diagnosed in animals that died before the scheduled sacrifice, this was demonstrated to also be

the case with the HCD. Furthermore, the haemolymphoreticular system was not a target of mefentrifluconazole in any of the repeated-dose toxicity studies. There was thus no indication that mefentrifluconazole induced malignant lymphoma of the haemolymphoreticular system nor speeded the progression of spontaneously-arising tumours. The DS did not consider these tumours relevant for classification.

Regarding the uterine adenocarcinomas in females, the DS noted that they did not show a dose-response relationship, with the incidence in the low-dose group (18 %) being higher than that in the high-dose group (10 %). The incidences in all the treatment groups were also well within the relevant HCD (12 – 28 %, mean 18.8 %) for this common tumour of aged female rats. The DS thus concludes that the increased incidence above the concurrent controls in the mefentrifluconazole study was incidental and not relevant for classification.

18-Month Carcinogenicity Study on Mice

The test was performed on C57BL/6JRj mice grouped as 50/sex/dose according to OECD TG 451. The animals were treated via the diet with doses slightly differentiated between sexes: 0, 20, 50, 200 ppm (equivalent to 0, 3.5, 9.1 and 36 mg/kg bw/day intake) for males and 0, 20, 50, 250 ppm (equivalent to 0, 4.9, 12.6 and 61.5 mg/kg bw/day intake) for females, respectively.

The DS concluded that there were no adverse effects at the low dose and no clinical signs in any group. At the mid-dose, changes in relative liver weight increase (18%) with hepatocellular fatty change in males (change in severity score from 2.0 in controls to 2.9 in the exposed group) were observed. At the highest dose, decreased mean body weight and body weight gain (up to -14% in males and -33% in females), increased relative liver weight (42% in males and 57% in females), increased incidence of fatty change and signs of (pre)degeneration in liver cells (eosinophilic inclusions in males and single cell necrosis in females), increased incidence of thyroid follicular cell hyperplasia in male only (74% vs 42% in controls).

The neoplastic findings consisted of follicular cell adenomas of the thyroid gland and hepatocellular adenomas and carcinomas of the liver.

With respect to the liver tumours, the DS concluded that mefentrifluconazole did not induce liver tumours in mice.

For the thyroid gland adenomas, the DS concluded that the tumours arose spontaneously. A dose-response relationship in the incidence of adenoma and follicular-cell hyperplasia was not evident in the females. In males, there was a statistically significant increase in the incidence of hyperplasia in the high-dose males (36 mg/kg bw/d), although again without a dose-response relationship. Notwithstanding, the DS considered that the increase in hyperplasia in the high-dose males was treatment-related and perhaps reflected an exacerbation of age-related thyroid changes. The increased incidence of hyperplasia was not associated with thyroid follicular-cell tumours in either the mefentrifluconazole-exposed groups or the historical control data. Overall, the DS concluded that the thyroid tumours were not relevant for classification.

Comments received during public consultation

One MS agreed with the argumentation and supported the DS’s proposal.

Assessment and comparison with the classification criteria

No human data were presented for evaluation.

The carcinogenicity potential of mefentrifluconazole was assessed in two species. Both studies were in conformity with OECD test guidelines and GLP compliant.

Assessment of carcinogenicity in rats

The neoplastic findings after 24 months of exposure to mefentrifluconazole in rats were limited to the high dose group when compared with the controls: slight increases in the incidence of malignant lymphoma (haemolymphoreticular system) in males and uterine adenocarcinoma in females.

Overall, the neoplasms findings were comparable between control and high-dose females: total number of primary neoplasms (73 vs. 65), benign neoplasms (62 vs. 53), and malignant neoplasms (11 vs. 12). In males, the total numbers of primary neoplasms (67 vs. 42), benign neoplasms (53 in controls vs. 35) and malignant neoplasms (14 in controls vs. 7) were higher in the control group than in the high-dose group. The total numbers of systemic and metastasised neoplasms were comparable between control and high-dose males and females.

The survival rate and incidences of tumours in the haemolymphoreticular system and uterus observed in rats are summarised in the following table:

	Males				**Females**			
Dose level [mg/kg bw/day]	**0**	**4**	**25**	**163**	**0**	**6**	**38**	**302**
No. of animals	50	50	50	50	50	50	50	50
Survival rate: *percent (absolute number of survivals)*	100% (50)	76% (38)	78% (39)	94% (47)	76% (38)	80% (40)	82% (41)	90% (45)
Haemolymphoreticular system *Number examined*	50	50	50	50	50	10	10	50
Lymphoma, malignant	0	2 (4%)	2 (4%)	3 (6%)	0	0	1 (2%)	0
Sarcoma, histiocytic	1	1	2	0	0	0	1	0
Uterus *Number examined*	—				50	38	37	50
Adenocarcinoma, endometrial					1 (2%)	7 (18%)	3 (8%)	5 (10%)
Schwannoma, malignant					0	1	1	1
Adenoma, endometrial					1	1	0	2

The applicant provided HCD for both tumours types from the same test facility and strain of rats for a period of 10/11 years before the test on mefentrifluconazole (period 2003 till 2013). However, the DS narrowed the time-frame of these data to studies that were contemporary to the mefentrifluconazole study (period 2013-2015). Both HCDs are summarised in the following table:

Tumour type	**Period 2003-2013**	**Period 2013-2015**
Malignant lymphoma	Mean: 2.5% (15/600)	Mean: 3.2% (8/250)
	Min.: 0% (0/50)	Min.: 0% (0/50)
	Max.: 6% (3/50)	Max.: 4% (2/50)
Adenocarcinoma, endometrial	Mean: 16.2% (97/600)	Mean: 18.8% (47/250)
	Min.: 2% (1/50)	Min.: 12% (6/50)
	Max.: 30% (15/50)	Max.: 28% (14/50)

RAC notes that the incidence of the malignant lymphomas in the high dose group in rats was at the uppermost boundary of the HCD for 10 years and slightly above (3/50 compared with 2/50) for the 5 year interval. However, no clear dose-response relationship could be identified for these tumours: neither the survival rate nor the differences between groups can support such a correlation.

The three animals in the high-dose group diagnosed with malignant lymphoma were decedents, as were the 2 animals in the low dose group. It is to be noted that the animals in the high dose group survived longer than those in the lower dose groups. In contrast, the two diagnosed animals in the mid-dose group survived to termination of the study. At the 12-month interim

sacrifice no case of malignant lymphoma was detected. In addition, the HCD shows that all but one animal with the same tumour type died before the scheduled sacrifice.

With regards to uterine adenocarcinomas in rats, RAC considers that the increased incidence did not show any dose-response relationship; moreover, it was within both the 10 and 5 years HCD in all the exposed groups. The survival rate in the high dose group (90%) was higher than the highest survival rate in the HCD for 5 years (88%). One of the high dose diagnosed females was a decedent; according to the data provided, this is a common occurrence also in the HCD.

RAC notes that many tumours were diagnosed in decedents before the scheduled sacrifice. However, the animals in the high dose group lived longer than those in the low-dose group. In addition, the two diagnosed animals in the mid-dose group survived until the termination of the study. In addition, since the haemolymphoreticular system was not a target in the repeated toxicity studies it is highly unlikely that the test substance is a tumour promoter. Therefore, RAC considers that the findings in rats do not support the hypothesis that the malignant lymphomas were induced by mefentrifluconazole.

Assessment of carcinogenicity in mice

In mice, the neoplastic findings consisted of follicular cell adenomas of the thyroid gland and hepatocellular adenomas and carcinomas of the liver as summarised in the table below:

Dose level [mg/kg bw/day]	**Males**				**Females**			
	0	**3.5**	**9.1**	**36**	**0**	**4.9**	**12.6**	**61.5**
No. of animals	50	50	50	50	50	50	50	50
Survival rate *percent (absolute number of survivors)*	98% (49)	96% (48)	100% (50)	100% (50)	94% (46/49)[a]	96% (46/48)[a]	95% (41/43)[a]	89% (42/47)[a]
THYROID exam.	50	50	50	50	50	50	50	50
Adenoma, follicular cell	0	0	0	2 (4%)	1 (2%)	0	1 (2%)	3 (6%)
LIVER exam.	50	50	50	50	50	50	50	50
Adenoma, hepatocellular	1 (2%)	2 (4%)	3 (6%)	0	0	0	0	0
Carcinoma, hepatocellular	1	1	1	0	1	0	0	0

[a] Some female mice were sacrificed for humane reasons, owing to skin lesions (a common spontaneous occurrence in this strain); these animals were disregarded for the calculation of survival. The figures reflect the survivors vs. the total number of animals in these particular cases.

The applicant provided HCD for thyroid tumours from the same test facility and strain of rats for a period of 10 years before the test on mefentrifluconazole (1998 till 2007). However, the DS narrowed the time-frame of these data to studies that were contemporary to the mefentrifluconazole study (dosing period 2013-2015). Both HCDs are summarised in the following table for males and females:

Tumour type	**Period 1998-2007**	**Period 2013-2015**
thyroid, follicular cell adenoma, Males	Mean: 1.5% (3/198)	Mean: 1.2% (3/250)
	Min.: 0% (0/50)	Min.: 0% (0/50)
	Max.: 2% (1/50)	Max.: 6% (3/50)
thyroid, follicular cell adenoma, Females	Mean: 2.6% (3/198)	Mean: 3.6% (9/250)
	Min.: 0% (0/50)	Min.: 0% (0/50)
	Max.: 6% (3/47)	Max.: 8% (4/50)

The significance of liver and thyroid tumours is detailed below.

Liver adenomas and carcinomas were present in the control, low and mid-dose groups but not in the high dose group of male mice. This finding indicates that the tumours are not treatment-related. All the male mice in the high dose group survived to the termination of the study; therefore, the absence of tumours cannot be attributed to the early death of the animals. In the groups of treated female mice, there were no hepatocellular adenomas. The sole type of tumour was a hepatocellular carcinoma detected in one female mouse in the control group. Therefore, RAC considers that the findings in mice do not support the hypothesis that the liver tumours were induced by mefentrifluconazole.

The incidence of the thyroid adenomas was slightly increased in the high dose groups of both males and females. However, when compared with the controls, the incidence was not statistically significant and was similar to the background incidences in this strain of mice. When compared with the HCDs, the incidence in males was within the upper range for the period 1998-2007 set of data but slightly exceeded the range for the test site data for the period 2013-2015; the excess consists of 1 animal in the high dose group. The incidence of thyroid gland adenomas in females was within (although at the upper range) the HCD for both data sets.

The hypothesis of the thyroid adenomas occurred as a continuum of treatment-related pathological consequences with a progression from hyperplasia was investigated. The Table below presents the incidences of thyroid follicular cell (multi)focal hyperplasia in both males and females treated with mefentrifluconazole. HCD are also presented below the Table:

	Males				**Females**			
Dose level [mg/kg bw/day]	**0**	**3.5**	**9.1**	**36**	**0**	**4.9**	**12.6**	**61.5**
No. of animals	50	50	50	50	50	50	50	50
THYROID examined	50	50	50	50	50	50	50	50
Hyperplasia, follicular cell, (multi)focal	21 (42%)	16 (32%)	17 (34%)	**37**** (74%)	19 38%	14 28%	**8*** 16%	26 52%

Statistical analysis: *: $p \leq 0.05$, **: $p \leq 0.01$ (Fisher's Exact test, 1-sided)
Historical control data (four 18-month studies in C57BL/6JRj mice, started: Jul-2013 to Mar-2014)
Males: mean: 31% (52/148); min.: 18% (9/50); max. 45% (22/49)
Females: mean: 18% (31/146); min.: 6% (3/50); max: 28% (13/47)
NOTE: In the male high dose group the incidence is within the corresponding HCD and in the mid-dose female group the incidence is less statistically significant than in the controls

RAC considers the relationship between thyroid follicular cell (multi)focal hyperplasia and thyroid tumours doubtful for the following reasons:

- in both males and females the incidence of follicular cell hyperplasia was not dose-related and stayed within HCD
- in one of two males and in two of three females, adenomas were reported in the absence of thyroid follicular-cell hyperplasia;
- no adenocarcinomas were detected in either sex
- the total number of neoplasms in all groups was comparable
- adenomas in the high-dose group did not result in early deaths
- no animal in any treatment groups had metastasis, whereas one was reported in a control female.

The observed increased incidence of thyroid follicular cell adenomas in mice was not statistically significant; the values were within the HCD for females and exceeded the data set by one animal in males. In females, there was no dose-response relationship for the adenoma and follicular cell hyperplasia. In males, there was also no dose-response relationship but a statistically significant increase (although within HCD) in the incidence of follicular cell hyperplasia could be seen in the high dose group. However, the increased incidence of hyperplasia was not associated with thyroid follicular cell tumours either in the mefentrifluconazole exposed groups or in the HCD. Therefore,

while it can be concluded that the cell hyperplasia in the high dose is treatment-related, RAC considers unlikely that mefentrifluconazole induced the thyroid tumours.

The observed liver adenomas and carcinomas in the males were not treatment-related since they were present in the control, low and middle dose groups but not in the high dose group. In females, the sole neoplastic finding was a hepatocellular carcinoma detected in one female mouse in the control group. Therefore, one can reasonably conclude that mefentrifluconazole did not induce liver tumours.

The database for the evaluation of mefentrifluconazole carcinogenicity is adequate and RAC bases its assessment on data from two animal carcinogenicity studies (1 rat and 1 mouse conventional cancer bioassays). The exposure route was oral in both the rat and the mouse studies.

Classification in category 1A concerns substances known to have carcinogenic potential for humans and is largely based on human evidence. Since there are no human data it cannot be concluded that mefentrifluconazole has known carcinogenic potential for humans; therefore Category 1A is not applicable.

Category 1B is for substances presumed to have carcinogenic potential for humans. Classification is largely based on animal evidence. Following an overall evaluation of the human evidence and the tumour data from one rat and one mouse bioassay, it is concluded that there is not sufficient evidence for carcinogenicity and a classification of mefentrifluconazole in category 1B is thus not warranted. The evaluation of strength of evidence and additional considerations including comparison with historical data is provided for each tumour type above.

Category 2 substances are suspected human carcinogens. Classification is based on evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations. There is insufficient evidence to support a classification in category 2 based on the evaluation of the rat study. The neoplastic findings at 24 months (malignant lymphoma (haemolymphoreticular system) in males and uterine adenocarcinoma in females) are not considered related to treatment. In the mouse study, two tumour types were considered in detail. In mice, the neoplastic findings consisted of follicular cell adenomas of the thyroid gland and hepatocellular adenomas and carcinomas of the liver. The observed increased incidence of thyroid follicular cell adenomas in mice was not statistically significant and generally within the HCD for females and exceeded the data set by one animal in males. RAC considers unlikely that mefentrifluconazole induced the thyroid tumours. The observed liver adenomas and carcinomas in the males were not treatment-related since they were present in the control, low and mid-dose groups but not in the high dose group. Therefore, RAC can reasonably conclude that mefentrifluconazole did not induce liver tumours. There is insufficient evidence to support a classification in category 2 based on the mouse data.

In conclusion, the evaluated data show that mefentrifluconazole does not meet the classification criteria for carcinogenicity under CLP and RAC agrees with the DS proposal for **no classification**.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The reproductive toxicity potential of mefentrifluconazole was investigated in one study for adverse effects on sexual function and fertility and two studies for developmental effects. All the studies are guideline and GLP compliant.

Sexual function and fertility

In a two-generation reproduction toxicity study (OECD TG 416), mefentrifluconazole was administrated in the diet to 25/sex/dose Wistar rats in the dose levels of 0, 25, 75 and 200 mg/kg bw/day. The animals were mated according to the dose groups to produce the F_1 and F_2 generations. The test substance was administered continuously throughout the study.

Overall, the DS concluded that a specific effect on fertility, reproduction and pregnancy outcome was not demonstrated by this study. Slight changes in some of the reproduction parameters (which were, moreover, within the historical control ranges), toxicity to offspring and delayed development were evident only at a dose that also resulted in parental toxicity (decreased food consumption and body weights), with an apparent lack of maternal care. Therefore, mefentrifluconazole did not show evidence of specific reproductive toxicity in this study. In accordance with the CLP criteria, classification for effects on fertility and reproductive performance may be warranted if adverse effects are observed in the absence of other toxic effects, or if occurring together with other toxic effects, the adverse effect on reproduction is considered not to be a secondary consequence of the other toxic effect. On this basis, the DS did not propose classification for adverse effects on sexual function and fertility.

Studies on developmental toxicity

Mefentrifluconazole was investigated for potential developmental toxicity by means of an OECD TG 414 *Prenatal Developmental Toxicity Study* test protocol in rats as well as in rabbits.

A guideline-compliant developmental toxicity study has been conducted in rats at doses of mefentrifluconazole up to 400 mg/kg bw/d, administered daily from implantation to one day prior to the expected day of parturition (GD 6-19). At this dose, maternal toxicity was evident as reduced food consumption, body weight and body-weight gain. There were no indications of maternal toxicity at the low- and mid-doses of 50 and 150 mg/kg bw/d.

The reproduction data were comparable between all the groups; hence, there was no evidence that mefentrifluconazole resulted in the deaths of embryos or foetuses. There was also no consistent evidence that the test substance affected foetal weights. Two foetuses had multiple malformations, one in the mid-dose group and one in the high-dose group. The malformations in each foetus were different and thus a relationship to mefentrifluconazole exposure was not established. Other malformations were distributed equally across the groups and so, likewise, were not associated with the test substance.

According to the DS, there was no treatment-related effect on the incidence of visceral malformations or variations in this study, nor on skeletal malformations. Slight, statistically significant, increases above the concurrent controls were noted in the incidence of two skeletal variations, supra-occipital hole and misshapen sacral vertebrae. Both findings were, nevertheless, within the historical control ranges, which showed them to be common findings; additionally, in the case of the former, the incidence was not dose related. There was, therefore, not a clear relationship between these findings and exposure to mefentrifluconazole.

The developmental toxicity of mefentrifluconazole in rabbits has been investigated in a study in which the test substance was administered orally at doses up to 25 mg/kg bw/d from the time of implantation to one day prior to the expected day of parturition (GD 0-28).

The highest dose in this study was chosen on the basis of two range-finding studies in non-pregnant female rabbits, in which 2 / 3 animals dosed with 50 mg/kg bw/d and 1 / 3 animals dosed with 25 mg/kg bw/d mefentrifluconazole were sacrificed because of poor condition, no or reduced faeces and almost zero food consumption, such that they lost weight throughout the study (21 days' duration). No treatment-related adverse effects occurred in a second range-finding study when 20 mg/kg bw/d was administered to pregnant rabbits. Hence, the study

authors determined 50 mg/kg bw/d to be a lethal dose, with 25 mg/kg bw/d representing half this dose. This dose in the main study did not induce signs of maternal toxicity: there were no overt clinical signs, and food consumption, body weight, body-weight gain and carcass weights were unaffected. The requirement of the test guideline that 'the highest dose should be chosen with the aim to induce some developmental and/or maternal toxicity (clinical signs or a decrease in body weight)' was thus not met. However, the difficulty of selecting appropriate doses for developmental toxicity studies is acknowledged, particularly in rabbits, which are susceptible to abortion and death when food intake is drastically reduced (Matsuoka *et al*., 2006).

In the main study, doses of mefentrifluconazole up to 25 mg/kg bw/d did not affect reproduction parameters or fetal weights, and thus did not exhibit any embryo- or fetal-toxicity. There were no statistically significant or dose-related increases in any type of malformation, variation, or unclassified observation. The total numbers of malformations and variations were lower than or the same as the control values.

In conclusion, the DS concluded that mefentrifluconazole was not a developmental toxicant in rats or rabbits under the conditions of the available studies.

Adverse effects on or via lactation

The DS did not propose a classification via lactation.

Comments received during public consultation

Three comments were received during public consultation. Two MS were in favour of no classification and one proposed classification of mefentrifluconazole as Repr. 2; H361f. Fertility was considered affected by this MS due to the reduction of implantation sites in the F_1 parents in the two generation study on rats. The same MS noted that this reduction was seen in the absence of pronounced parental toxicity.

Assessment and comparison with the classification criteria

Fertility

The potential adverse effects of mefentrifluconazole on the integrity and performance of the male and female reproductive systems, the oestrus cycle, mating behaviour, conception, gestation, parturition, lactation and the growth and development of the offspring were assessed in a guidance-compliant two generation study on rats.

The parental toxicity was not present at the low dose of 25 mg/kg bw/day. At the mid-dose of 75 mg/kg bw/day parental toxicity was detected as slight liver dysfunction. At the high dose of 200 mg/kg bw/day the parental toxicity was evident as consistent reduction of food consumption, body weights and body weight gain, liver cell hypertrophy and altered clinical-chemistry parameters. Also, at this dose there were two does with insufficient nursing and stillborn pups respectively.

The male and female mating index was 100 % in all groups in both generations. The fertility index was 100% in the F_0 parents but slightly lower at the high dose F_1 generation. The value was lowered as a result of two does which had no implants; however the value was within the HCD and there were no pathological findings. Therefore, RAC considers that this finding is not associated with the treatment with mefentrifluconazole.

In the high dose F_1 generation, there were also some additional reductions of reproductive parameters. There was a decrease of implantation sites per dam, with a consequent reduction in pups delivered and a slight increase in the post-implantation losses. These reductions were still

within the HCD. However, two F_1 females at the high dose (200 mg/kg bw) did not deliver any live pups: one delivered six still-born pups, whilst the other female showed evidence of just one implantation site but no pups. These two females caused the slight reduction in the gestation index. However, this decrease was not statistically significant and fitted within the HCD. In summary, since the post-implantation losses in the high-dose group in both generations were similar to the concurrent and historical controls, the findings were not attributed to the treatment with mefentrifluconazole.

In the F_0 parent females, the duration of the gestation was increased by half a day; this increase was statistically significantly, but not considered adverse. In the F_0 generation an abnormality in parturition was noted: one dam delivered on GD 23 and had total litter loss on PND 2. However, this was a single occurrence and there is no information to link the litter loss to the delay in parturition; consequently, it was not considered treatment-related. The mean duration of gestation was not affected in F_1 dams but there was one dam with delayed delivery: although on GD 22 it appeared to be in normal condition, on GD 23 it showed severe deterioration in general condition and was unable to deliver; finally on GD 24 it delivered only still-born pups. As in the case of the F_0 generation it was an isolated abnormality that was not considered to be associated with treatment.

The viability index did not show a dose-dependent trend and was not statistically significantly modified in any group. The slight reductions in the high-dose groups of both generations arose from a single whole litter loss. This finding was associated with the maternal toxicity evident at this dose: lower food consumption and lower body weights early in lactation affected the development of the pups.

The increased incidence of dilated renal pelvis in the high-dose group of the F_2 generation may be attributed to the treatment as suggested by the DS. However, the incidence did not show a dose-effect trend and was associated only with the high-dose at which maternal toxicity was evident. Therefore, it was not considered as evidence of a developmental consequence of exposure to mefentrifluconazole.

The sexual maturation was not affected by the treatment with mefentrifluconazole; the slight delay detected in the high dose group was within the HCD.

In summary, exposure to mefentrifluconazole did not induce specific treatment-associated effects in rats. The noted variations in some of the reproduction parameters, offspring toxicity and delayed development were evident only at doses that resulted in parental toxicity with associated lack of maternal care.

Development

The potential adverse effects of mefentrifluconazole on development were assessed in a guidance compliant study in two species: rats and rabbits.

In rats, the maternal toxicity was observed only at the high dose in terms of reduced food consumption, body weight and body weight gain. There were no deaths or clinical signs of toxicity in any group.

There were no dose-related differences between control and test groups in the mean number of pre- and post-implantation losses, the number of resorptions and viable foetuses. The mean placenta weights at the high dose was slightly increased but was within the HCD and was not associated with impaired foetal development.

The mean foetal weights were not affected except for a decrease only in females at the high dose; however the value was within HCD.

The total incidences of external, visceral and skeletal results showed no statistically significant differences. However, some skeletal variations, such as supra-occipital holes and misshapen sacral vertebra, appeared statistically significantly increased at the mid and higher dose but the values were within the associated HCD and a clear increase with the dose was not seen. On the other hand, the dumb-bell ossification reported as foetal incidence or affected foetuses/litter show some increase with the dose but the results were not statistically significant. The severity and incidence of these variations cannot clearly be attributed to the exposure and thus do not provide evidence of developmental toxicity. Also, dilated renal pelvis was noted but the values were also within the range of the HCD.

In rabbits, the maternal toxicity was not evident in all groups; the slight and isolated modifications in two parameters of clinical chemistry and globulin levels in the high dose appear as treatment related but were adaptive rather than toxic.

The mean foetal weights were fully comparable among all groups. Overall, all the types of malformations, variations and/or unclassified observations recorded did not show dose-related increases or were not statistically significant.

Adverse effects on or via lactation

The lactation index indicated that pup survival between PND 4 and 21 was high in all the groups. The slight reduction in the viability index at 200 mg/kg bw/day was a consequence of a single complete litter loss. This appeared to be the result of inadequate nursing which in turn was the consequence of reduced food intake by the dam with entire litter loss. In general, this food reduction during lactation may have affected the development of the pups. At this dose, body weight of pups was reduced in each generation when compared with the concurrent controls; however, as previously stated, the survival rate was high. There was no toxicokinetics data of the presence of mefentrifluconazole or its metabolites in the breast milk. Consequently, it is unknown whether this finding can be attributed or not to the presence of the test substance in the breast milk. Overall, RAC concludes that mefentrifluconazole is not seen as producing effects on or via lactation and no specific classification is proposed in agreement with the DS.

Conclusions on reproductive toxicity

The observed effects in the fertility and developmental studies were either not statistically significant, did not show a dose-dependent trend, or the effects were within the HCD. Therefore, RAC considers that they do not warrant classification and agrees with the proposal of the DS for **no classification** as a reproductive toxicant.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The DS proposed to classify the substance as Aquatic Acute 1; H400 (M=1) based on a 96h mean measured EC_{50} value of 0.532 mg/L for the fish *Oncorhynchus mykiss*, and as Aquatic Chronic 1; H410 (M=1) based on lack of rapid degradation and a 21d nominal EC_{10} value of 0.0175 mg/L for *Daphnia magna*.

Degradation

Mefentrifluconazole was hydrolytically stable (< 10% degradation) in buffer solutions at pH 4, 5, 7 and 9 in a 30 days OECD TG 111 test at 25°C in the dark.

It was observed to degrade rapidly inan aqueous photolysis study (OECD TG 316, GLP) with > 95% degradation occurring after 15 days. Four major photolytic degradants were detected (i.e. degradants that were detected in concentrations > 10%, > 5% at two consecutive time points or > 5% and increasing at study termination). A DT_{50} value of 2.3 days was calculated for mefentrifluconazole by SFO[1] kinetics. The DS noted that photolysis is of uncertain relevance as a route of degradation in typical European aquatic environments and, given the available data, there is insufficient information to evaluate photodegradation in terms of mineralisation or transformation to non-classifiable substances. Therefore, aquatic photolysis is not considered further in relation to fulfilling the criteria for rapid degradation.

A ready biodegradation test according to OECD TG 301B (CO_2 Evolution (Modified sturm Test)) using municipal activated sludge resulted in no degradation after 28 days at 22°C. The substance is therefore not readily biodegradable.

Furthermore, no significant degradation was observed in an aerobic mineralisation study (OECD TG 309, GLP) (<1 0% Applied Radioactivity (AR) mineralised after 63 days at 20°C).

An aerobic water/sediment degradation study was performed at 20°C in the dark (OECD TG 308, GLP). Two natural water/sediment systems of differing organic carbon content were sampled in November 2013 for the chlorophenyl and triazole radiolabelled experiments and in May 2014 for the trifluoromethylphenyl radiolabelled experiment. The study has shown that mefentrifluconazole partitions rapidly from the water phase to the sediment phase (water geometric mean $DissT_{50}$ 1.5 days, $DissT_{90}$ 24.0 days). After 100 days < 5% AR was detected as not degraded parent compound in the water phase with > 45% AR detected in the sediment phase of both systems. Two major degradants were detected in the test systems: 1,2,4-triazole (10.2% AR) and M750F003 (5.9% AR). The maximum amount of mineralisation to CO_2 observed was 5.1% after 100 days. For the total system, $DegT_{50}$ values of 122.2 and 213.1 days were calculated (at 20°C, SFO kinetic fit) for mefentrifluconazole (geometric mean 163.4 days).

The DS concluded that degradation information does not provide sufficient data to show that mefentrifluconazole is ultimately degraded to > 70% within 28 days (equivalent to a half-life of less than 16 days), or can be transformed to non-classifiable products. Consequently, the DS considered mefentrifluconazole not rapidly degradable for the purpose of classification and labelling.

Bioaccumulation

The measured octanol-water partition coefficient (log K_{OW}) is 3.4 at 20°C and it is not pH dependent.

A fish bioaccumulation study (OECD TG 305, GLP) is available for mefentrifluconazole. Rainbow Trout (*Oncorhynchus mykiss*) was exposed to a single concentration (0.010 mg/L) of the mixture of radiolabelled ^{14}C-mefentrifluconazole and unlabelled test substance (ratio 2:1) for 14 days in a flow-through system, followed by a 7 days depuration period. The BCF (growth corrected and lipid-normalized) for whole fish was 385 L/kg. Additionally, rapid depuration of

[1] Single First-Order Rate Model

mefentrifluconazole was observed with a depuration half-life of 0.60 days (growth corrected, based on total radioactivity). The DS concluded that mefentrifluconazole does not have potential to bioaccumulate in aquatic organisms.

Aquatic toxicity

Reliable aquatic toxicity data are available for all three trophic levels, and a summary of the relevant information is provided in the following table (the key endpoints used for the classification are highlighted in bold). No information is available to the RAC regarding the different toxicity of the optical isomers. Some references are not cited in the Table below because they were claimed as confidential.

The DS provided a short note regarding four existing studies performed on sediment-dwelling invertebrates (*Chironomus riparius, Chironomus dilutes*, *Hyalella Azteca* and *Leptocheirus plumulosus*). The results of the studies were not used for the classification because the EC_{50} and NOEC values were only presented in relation to sediment concentrations of mefentrifluconazole (mg/kg); no mg/L endpoints were available.

Summary of relevant information on aquatic toxicity

Method/Exposure	**Test organism**	**Endpoint**	**Toxicity values in mg/L**	**Reference**
Short-term toxicity to fish				
OECD TG 203 Flow through	*Oncorhynchus mykiss*	96 h LC_{50} (mortality)	**0.532 mm**	Anonymous (2014)
OECD TG 203 Static	*Danio rerio*	96 h LC_{50} (mortality)	0.906 mm	Anonymous (2015)
OECD TG 203 Semi-static	*Cyprinodon variegatus*	96 h LC_{50} (mortality)	0.761 mm	Anonymous (2014)
OECD TG 203 Flow through	*Cyprinus carpio*	96 h LC_{50} (mortality)	1.126 mm	Anonymous (2015c)
Long-term toxicity to fish				
Early life stage OECD TG 210 Flow through	*Danio rerio*	36 d NOEC[1] growth (body length)	0.027 mm	Anonymous (2015a)
Sexual development test OECD TG 234 Flow through	*Danio rerio*	69 d NOEC[1] (hatching success, survival, toxic signs, body weight, body length, maturity, sex ratio, gonad histopathology and vitellogenin)	≥ 0.045 mm	Anonymous (2015b)
Early life-stage EPA 850.1400 Flow through	*Cyprinodon variegatus*	35 d NOEC[1] (toxic signs)	0.147 mm	Anonymous (2015)
Short-term toxicity to aquatic invertebrates				
OECD TG 202 Static	*Daphnia magna*	48 h EC_{50} (immobility)	0.944 mm	Brzozowska (2014a)
EPA 850.1035 Flow through	*Americamysis bahia*	96 h LC_{50} (mortality)	1.3 mm	VanHooser (2014a)

Method/Exposure	Test organism	Endpoint	Toxicity values in mg/L	Reference
Short-term toxicity to fish				
EPA 850.1025 Flow through	*Crassostrea virginica*	96 h EC_{50} (shell growth inhibition)	0.9472 mm	VanHooser (2015a)
Long-term toxicity to aquatic invertebrates				
OECD TG 211 Semi-static	*Daphnia magna*	21-d EC_{10} (reproduction (offspring per female))	**0.0175 n**	Janson (2014a)
		21-d NOEC (reproduction (offspring per female))	**0.010 n**	
EPA 850.1350 Flow through	*Americamysis bahia*	28-d NOEC[1] (survival, offspring per female, days to first brood release, length and dry weight)	≥ 0.0132 mm	Dinehart (2016a)
OECD TG 211 Semi-static	*Daphnia pulex*	21-d EC_{10} (reproduction (offspring per female))	0.0573 n	Janson (2015a)
		21-d NOEC (reproduction (offspring per female))	0.0282 n	
OECD TG 211 Semi-static	*Daphnia longispina*	21-d EC_{10} (reproduction (offspring per female))	0.0558 n	Janson (2015b)
		21-d NOEC (reproduction (offspring per female))	0.0338 n	
Toxicity to algae and aquatic plants				
OECD TG 201 (2011) Static	*Pseudokirchneriella subcapitata*	72-h E_rC_{50}	1.352 mm	Brzozowska (2014b)
		72-h E_rC_{10} 72-h NOE_rC	0.904 mm 0.209 mm	
EPA 850.4500 Static	*Skeletonema costatum*	72-h E_rC_{50}	0.679 mm	Bergfield (2015a)
		72-h E_rC_{10} 72-h NOE_rC	0.373 mm 0.0985 mm	
EPA 850.4500 Static	*Navicula pelliculosa*	72-h E_rC_{50}	1.347 mm	Bergfield (2015b)
		72-h E_rC_{10} 72-h NOE_rC	0.478 mm 0.303 mm	
EPA 850.4500 Static	*Anabaena flos-aquae*	72-h E_rC_{50}	> 3.08 mm	Bergfield (2015c)
		72-h E_rC_{10} 72-h NOE_rC	> 3.08 mm ≥ 3.08 mm	
OECD TG 221 (2006) Static	*Lemna gibba*	7-d E_rC_{50}	> 2.017 im	Swierkot (2014a)
		7-d E_rC_{10} 7-d NOE_rC	> 2.017 im ≥ 2.017 im	

[1]The NOEC is given as primary endpoint, since no dose-response relationship was derived from the study which could be used for ECx calculations.

Acute and long-term aquatic toxicity data on mefentrifluconazole are available for fish, invertebrates, algae and aquatic plants. From the available aquatic toxicity data, fish are the most sensitive trophic group. The lowest acute endpoint was a 96h mean measured EC_{50} of 0.532 mg/L, reported for *Oncorhynchus mykiss*. The results of long-term aquatic toxicity studies indicate that the invertebrate are the most sensitive taxon and the lowest chronic value is 21d nominal NOEC of 0.01 mg/L for *Daphnia magna*. In the same study, the 21d nominal EC_{10} for *D. magna* (based on the same reproduction endpoint) was 0.0175 mg/L. In this study, all analytical measurements were between 81-97% of nominal concentrations.

Comments received during public consultation

Three MSCAs provided public comments, and all agreed with the proposed classification for environmental hazards.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS proposal to consider mefentrifluconazole as not rapidly degradable. The substance is hydrolytically stable at environmentally relevant pHs (pH 5-9) and is not readily biodegradable. No significant degradation in the aerobic mineralisation study was observed. The results of water/sediment simulation study show that mefentrifluconazole dissipates rapidly from the water phase to the sediment phase (water geometric mean DissT50 1.5 days, DissT90 24.0 days). Mineralisation of mefentrifluconazole was low, reaching a maximum of 5.1% after 100 days. Total system DegT50 values were of 122.2 and 213.1 days, with a geometric mean value of 163.4 days (at 20°C).

Bioaccumulation

RAC agrees with DS that mefentrifluconazole has a low potential to bioaccumulate in aquatic organisms. The basis for this is the measured whole fish BCF value of 385 L/kg being below the decisive CLP Regulation criterion of 500. Additionally, a Log K_{OW} value of 3.4 is below the CLP Regulation threshold of 4.

Aquatic toxicity

Reliable short-term aquatic toxicity data are available for all three trophic levels and the lowest 96h EC_{50} value (mean measured) is 0.532 mg/L for the fish *Oncorhynchus mykiss*. As this concentration is below the threshold value of 1 mg/L, RAC concludes that a classification as Aquatic Acute 1 (H400) is justified. As $0.1 < EC_{50} \leq 1.0$ mg/L, the acute M-factor is 1.

Reliable long-term aquatic toxicity data are available for all three trophic levels. The lowest values were found in a *Daphnia magna* study: nominal 21 d NOEC and EC_{10} values of 0.01 mg/L and 0.0175 mg/L, respectively. As both concentrations are below the threshold value of 0.1 mg/L for not rapidly degradable substances, RAC concludes that a classification as Aquatic Chronic 1 (H410) is justified. The DS pointed out that the choice of chronic M-factor depends on whether the NOEC (0.01 mg/L) or EC_{10} (0.0175 mg/L) for *D. magna* is chosen as the classification ranges are $0.001 < NOEC \leq 0.01$ (M=10) or $0.01 < NOEC \leq 0.1$ (M=1). In line with the current CLP Guidance (Version 5.0, July 2017), the DS gave preference to the EC_{10} over the NOEC value and therefore proposed a chronic M-factor of 1.

RAC notes that the EC_{10} value is almost twice higher than the NOEC value. However, RAC considers more appropriate to use EC_{10} value for aquatic chronic classification and for M-factor

derivation because the NOEC values strongly depends on the experimental design (number of doses, width of the inter-dose interval, etc.), whereas EC_{10} value is derived from the whole concentration-response curve.

In summary, RAC supports the DS's proposal that mefentrifluconazole should be classified as **Aquatic Acute 1 (H400)** with an **M-factor** of **1** and as **Aquatic Chronic 1 (H410)** with an **M-factor** of **1**.

ANNEXES:

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

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