Committee for Risk Assessment

RAC

Annex 1

Background document

to the 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

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

Adopted

9 March 2018

CLH report

Proposal for Harmonised Classification and Labelling

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

MEFENTRIFLUCONAZOLE

EC Number: –

CAS Number: 1417782-03-6

Index Number: –

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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON ON (2RS)-2-[4-(4-CHLOROPHENOXY)-2-(TRIFLUOROMETHYL)PHENYL]-1-(1H-1,2,4-TRIAZOL-1-YL)PROPAN-2-OL; MEFENTRIFLUCONAZOLE

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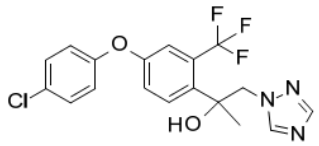
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	(2RS)-2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-1-(1H-1,2,4-triazol-1-yl)propan-2-ol
Other names (usual name, trade name, abbreviation)	BAS 750 F
ISO common name (if available and appropriate)	Mefentrifluconazole (ISO provisionally assigned)
EC number (if available and appropriate)	not assigned
EC name (if available and appropriate)	not assigned
CAS number	1417782-03-6
Other identity code (if available)	BASF Reg.No. 5834378
Molecular formula	$C_{18}H_{15}ClF_3N_3O_2$
Structural formula	
SMILES notation (if available)	CC(O)(Cn1cncn1)c3ccc(Oc2ccc(Cl)cc2)cc3C(F)(F)F
Molecular weight or molecular weight range	397.783 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Racemic mixture
Degree of purity (%) (if relevant for the entry in Annex VI)	Minimum purity: 96.5%

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
BAS 750 F CAS No.: 1417782-03-6	96.5 – 99.5%	not assigned	Skin Sens. 1B, H317 Aquatic Acute 1, H400 Aquatic Chronic 1, H410

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

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
Confidential	-	-	-	-

There are a number of process impurities identified in the substance. These have been taken into account and are not considered to impact on the classification proposed in this dossier. Further information on the impurities is considered confidential, but full details are provided in the IUCLID.

Current CLH in Annex VI of CLP: One of the substances has an existing entry in Annex VI of CLP. However, given the concentration at which this impurity is present and the available data on mefentrifluconazole, this is not considered to impact on the classification proposed in this dossier. Full information is provided in the IUCLID.

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

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
None	-	-	-	-	-

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

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used
-	-	-	*The substance used in the studies is considered equivalent to that outlined above. The purity of the tested batch is specified in each section below.*	-

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	New active substance, not yet listed in Annex VI										
Dossier submitter's proposal	not assigned	Mefentrifluconazole (ISO) : (2RS)-2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-1-(1H-1,2,4-triazol-1-yl)propan-2-ol	not assigned	1417782-03-6	Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H317 H400 H410	Wng GHS07 GHS09	H317 H410		M = 1 (acute) M = 1 (chronic)	
Resulting Annex VI entry if agreed by RAC and COM	not assigned	Mefentrifluconazole (ISO) : (2RS)-2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-1-(1H-1,2,4-triazol-1-yl)propan-2-ol	not assigned	1417782-03-6	Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H317 H400 H410	Wng GHS07 GHS09	H317 H410		M = 1 (acute) M = 1 (chronic)	

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

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

Hazard class	Reason for no classification	Within the scope of public consultation
Hazardous to the aquatic environment	**Harmonised classification proposed**	Yes
Hazardous to the ozone layer	Data conclusive but not sufficient for classification	Yes

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Mefentrifluconazole is a new active substance under Regulation 1107/2009 and has not previously been evaluated.

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.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Mefentrifluconazole is a new active substance under Regulation 1107/2009.

5 IDENTIFIED USES

Mefentrifluconazole is a new fungicidal active substance for use in plant protection products.

6 DATA SOURCES

Mefentrifluconazole is a new active substance under Regulation 1107/2009 and has not been placed on the market yet. The present evaluation exclusively relies on data submitted in the context of the application for approval as an active substance under Regulation 1107/2009.

At the time of submission, the substance is not registered under REACH.

7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Test material (Batch no., purity)
Physical state at 20°C and 101,3 kPa	white, solid and odourless crystalline powder	Kroehl, 2014a	L84-238: 99.7%
	fine powdered, off-white solid of moderate thiolic odour	Kroehl, 2014b	COD-001740: 98.8%
Melting/freezing point	Melting point (onset): 126 °C Decomposition temp. (onset): approx. 300 °C (onset of exothermic peak)	EU A1 (DSC) Kroehl, 2014a	L84-238: 99.7%
	Melting point (onset): 125 °C Decomposition temp. (onset): approx. 300 °C (onset of exothermic peak)	Kroehl, 2014b	COD-001740: 98.8%
Boiling point	n.a. (decomposition at approx. 300 °C)	–	–
Relative density	n.a. (solid)	–	–
Vapour pressure	Vapour pressure: $p = 3.2 \cdot 10^{-6}$ Pa (20 °C) $p = 6.5 \cdot 10^{-6}$ Pa (25 °C)	EU A4 Kroehl, 2014a	L84-238: 99.7%
	Henry Constant: $H = 1.6 \cdot 10^{-3}$ Pa · m^3 · mol^{-1}	Kroehl, 2014c	calculated
Surface tension	Not applicable, substances with a water solubility < 1 mg/L need not be tested.	–	–
Water solubility	water: 0.81 mg/L (pure water, resulting pH value: 6.8) pH 4: 0.66 mg/L (acetate buffer) pH 7: 0.71 mg/L (phosphate buffer)	EU A6 (column elution) Wilbrand, 2013a	L84-283: 99.7%
Partition coefficient n-octanol/water	Results determined at 20 °C pH 4*: log P_{OW} = 3.4 pH 7: log P_{OW} = 3.4 pH 7*: log P_{OW} = 3.3 pH 9*: log P_{OW} = 3.4 * buffered	EU A8 (HPLC) Wilbrand, 2013c	L84-238: 99.7%
Flash point	Not applicable (melting point > 40 °C).	Moeller, 2014	COD-001740: 98.8%
Flammability	Flammability: No ignition of the test substance by flame in the preliminary test (the test substance melted). Thus, the main test was omitted. Relative self-ignition of solids: Test not performed (melting point < 160 °C).	EU A10 (comparable to Test N1 of the UNRTDG Manual of Tests and Criteria) Moeller, 2014	COD-001740: 98.8%

Property	Value	Reference	Test material (Batch no., purity)
Explosive properties	Thermal Stability (DSC method): 1st reaction: onset 110 °C, energy intake 110 J/g (endothermic) 2nd reaction: onset 340°C, energy release 580 J/g (exothermic) Mechanical sensitivity, friction: No reaction observed in six tests using BAM friction apparatus with a force of 360 N. Mechanical sensitivity, impact. No reaction observed in six tests using BAM drop hammer (mass 10 kg, drop height 40 cm). Thermal sensitivity, Koenen-test: The results of the test series with a 2.0 mm diameter orifice plate showed no explosion. Therefore, further tests were not required. Final conclusion: not explosive	EU A14 (comparable to the test methods in Part I of the UNRTDG Manual of Tests and Criteria) Moeller, 2014	COD-001740: 98.8%
Self-ignition temperature	Test not performed (melting point < 160 °C).	–	–
Oxidising properties	The highest burning rate of a test mixture of test substance with cellulose (1.07 mm/s) is lower than the highest burning rate of a mixture of barium nitrate with cellulose (1.27 mm/s). Conclusion: Not oxidizing.	EU A17 (comparable to Test O1 of the UNRTDG Manual of Tests and Criteria) Moeller, 2014	COD-001740 : 98.8%
Granulometry	No data		
Solubility in organic solvents	Results (in g/L) were obtained at 20 °C (± 0.5 °C) applying the flask method. Acetone 93.2 (± 1.6) Ethyl acetate 116.2 (± 1.8) Methanol 73.2 (± 3.2) 1,2-Dichloroethane 55.3 (± 0.4) Acetonitrile 49.4 (± 0.7) Xylene 8.5 (± 0.1) n-Heptane $9.46 \cdot 10^{-2}$ ($\pm 0.9 \cdot 10^{-3}$)	Wilbrand, 2013c	COD-001740: 98.8 %

Property	Value	Reference	Test material (Batch no., purity)
Dissociation constant	Due to the low solubility of the test substance in water, the titration method is not suitable for the determination of the dissociation constant. Instead of the titration method the spectroscopic method was used in this study. pK_a at 20 °C: 2.7 ± 0.5 pK_a at 30 °C: 2.5 (± 0.5) pK_a (calculated; ACD Lab 12.01): 3.0 Due to the high variation of the test results within each measurement as well as to the high relative standard deviations, the pKa value calculated is reported as the final result of the dissociation constant of Reg. No. 5834378.	Wilbrand, 2013d	L84-238: 99.7 %
Viscosity	n.a.	–	–

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 9: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
OECD 113 EU A.14	not explosive	–	Moeller M. 2014

8.1.1 Short summary and overall relevance of the information provided on explosive properties

The explosive properties of mefentrifluconazole have been tested according to OECD 113 (EU A14) which is comparable to the test methods in Part I of the UNRTDG Manual of Tests and Criteria. DSC showed two reactions (1st reaction: onset 110 °C, energy intake 110 J/g (endothermic); 2nd reaction: onset 340°C, energy release 580 J/g (exothermic)). No reaction observed in six tests using BAM friction apparatus with a force of 360 N. No reaction observed in six tests using BAM drop hammer (mass 10 kg, drop height 40 cm).

A thermal sensitivity (Koenen) test showed no explosion in a test series with a 2.0 mm diameter orifice plate. Therefore, further tests were not required.

8.1.2 Comparison with the CLP criteria

A substance is considered for classification as an explosive substance where a positive result is obtained in the test series indicated in figure 2.1.2 of Annex I of the CLP regulation. Mefentrifluconazole was not found to be sensitive to the effects of heat, shock or friction. Consequently, it does not meet the criteria for classification as an explosive substance.

8.1.3 Conclusion on classification and labelling for explosive properties

Not classified (conclusive but not sufficient for classification)

8.2 Flammable gases (including chemically unstable gases)

Not applicable.

8.3 Oxidising gases

Not applicable.

8.4 Gases under pressure

Not applicable.

8.5 Flammable liquids

Not applicable.

8.6 Flammable solids

Table10: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
EU A.10	No ignition of the test substance by flame in the preliminary test (the test substance melted). Thus, the main test was omitted	–	Moeller, 2014a

8.6.1 Short summary and overall relevance of the provided information on flammable solids

In a preliminary flammability test, no ignition of the test substance by flame was observed (the test substance melted). Thus, the main test was omitted.

8.6.2 Comparison with the CLP criteria

A substance (non-metal) is classified as a flammable solid when the burning time is < 45 seconds or the burning rate is > 2.2 mm/s. It is concluded that mefentrifluconazole is not highly flammable as it failed to ignite in the preliminary screening test and therefore does not meet the criteria for classification as flammable solid.

8.6.3 Conclusion on classification and labelling for flammable solids

Not classified (conclusive but not sufficient for classification).

8.7 Self-reactive substances

8.7.1 Short summary and overall relevance of the provided information on self-reactive substances

Test not performed (melting point < 160 °C).

8.7.2 Comparison with the CLP criteria

Substances or mixtures with a low melting point (< 160 °C) should not be considered for classification in this class since the melting process is endothermic and the substance-air surface is drastically reduced.

8.7.3 Conclusion on classification and labelling for self-reactive substances

Not classified (conclusive but not sufficient for classification).

8.8 Pyrophoric liquids

Not applicable.

8.9 Pyrophoric solids

8.9.1 Short summary and overall relevance of the provided information on pyrophoric solids

A study was not necessary due to practical experience in handling and use.

8.9.2 Comparison with the CLP criteria

The substance is known to be stable in contact with air at room temperature for prolonged periods of time, therefore the criteria for classification are not met.

8.9.3 Conclusion on classification and labelling for pyrophoric solids

Not classified (conclusive but not sufficient for classification).

8.10 Self-heating substances

8.10.1 Short summary and overall relevance of the provided information on self-heating substances

Test not performed (melting point < 160 °C).

8.10.2 Comparison with the CLP criteria

Substances or mixtures with a low melting point (< 160 °C) should not be considered for classification in this class since the melting process is endothermic and the substance-air surface is drastically reduced.

8.10.3 Conclusion on classification and labelling for self-heating substances

Not classified (conclusive but not sufficient for classification).

8.11 Substances which in contact with water emit flammable gases

8.11.1 Short summary and overall relevance of the provided information on substances which in contact with water emit flammable gases

No test is necessary since mefentrifluconazole does not contain metals or metalloids. Further experience in handling and use indicates that it will not emit flammable gases on contact with water.

8.11.2 Comparison with the CLP criteria

The substance does not meet the criteria for classification.

8.11.3 Conclusion on classification and labelling for substances which in contact with water emit flammable gases

Not classified (conclusive but not sufficient for classification).

8.12 Oxidising liquids

Not applicable.

8.13 Oxidising solids

Table 11: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
EU A.17	The highest burning rate of a test mixture of test substance with	–	Moeller, 2014a

Method	Results	Remarks	Reference
	cellulose (1.07 mm/s) is lower than the highest burning rate of a mixture of barium nitrate with cellulose (1.27 mm/s). Conclusion: Not oxidizing.		

8.13.1 Short summary and overall relevance of the provided information on oxidising solids

In a test for oxidising properties, a mixture of mefentrifluconazole with cellulose had a lower burning rate (1.07 mm/s) than a mixture of barium nitrate with cellulose (1.27 mm/s).

8.13.2 Comparison with the CLP criteria

A substance is classified as an oxidising solid when the burning time of a sample-to-cellulose mixture is less than or equal to the burning time of the appropriate reference sample. A mixture of mefentrifluconazole with cellulose had a lower burning rate than a mixture of barium nitrate with cellulose. Therefore, the criteria for classification are not met.

8.13.3 Conclusion on classification and labelling for oxidising solids

Not classified (conclusive but not sufficient for classification).

8.14 Organic peroxides

Not applicable, mefentrifluconazole does not contain peroxo moieties.

8.15 Corrosive to metals

No data.

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. Also, 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.

Supplemental information - In depth analyses by RAC

Explosive properties

The procedure of classification for explosive properties consists of two steps (see Supplemental information - In depth analyses by RAC). The proposed classification may only result from the conclusion drawn in the first step.

Step 1: screening procedure

According to CLP Regulation (Section **2.1.4.2**) the screening procedure is aimed at identifying the presence of such reactive groups and the potential for rapid energy release. If the screening procedure identifies the substance or mixture to be a potential explosive, the acceptance procedure from the UN RTDG has to be performed. Mefentrifluconazole has no previous indication of having explosive properties; however, to fully investigate this potential hazard, RAC applies the screening procedure.

A substance is not classified as explosive if it fulfils one of the following conditions:

a. There are no chemical groups associated with explosive properties, i.e. the molecule can react to produce very rapid increases in temperature and/or pressure. From its chemical structure, mefentrifluconazole has such a chemical group since two contiguous nitrogen atoms are present in the triazole ring. However, similar triazole compounds have been used for a long time and the triazole ring has not been shown to have explosive properties. Therefore, it cannot be unequivocally associated with explosive properties and from a practical point of view the condition is fulfilled;
b. The substance contains such chemical groups associated with explosive properties but the exothermic decomposition energy is less than 500 J/g and the onset of exothermic decomposition is below 500 ºC. It has to be underlined that the temperature limit is set to prevent the procedure being applied to a large number of organic materials which are not explosive but which will decompose slowly at above 500 ºC releasing more than 500 J/g in the process. Mefentrifluconazole was found to have a decomposition temperature of 300 ºC, which is below the cut-off value of 500 ºC. On the other hand, the measured decomposition energy of mefentrifluconazole (580 J/g) is higher than the indicated value of 500 J/g; RAC notes that this difference of 80 J/g is very small.

In summary, the screening procedure applied to mefentrifluconazole draws attention on two above conditions that needs consideration i.e. the triazole ring presents in the chemical structure and the decomposition energy. However, RAC notes that in practical terms these elements cannot be associated with explosive properties without some reservations.

Step 2: decision logic

The decision logic for classification is given in Annex I, Figure 2.1.2 of the CLP Regulation. Since mefentrifluconazole is neither manufactured with a view to using it for practical explosive or pyrotechnic effects, nor is it a candidate for ammonium nitrate emulsion, suspension or gel, the first question refers to the basic explosive properties investigated in Test series number 1. This test line-up answers the question "*Is it an explosive substance / mixture?*" based on three assays:

Type 1 (a): a shock test with defined booster and confinement to determine the ability of the substance to propagate a detonation (*UN Gap test*, zero gap);

Type 1 (b): a test to determine the effect of heating under confinement (Koenen test);

Type 1 (c): a test to determine the effect of ignition under confinement (time/pressure test).

Mefentrifluconazole was not subjected to type 1(a) test (UN Gap test, zero gap); however, according to the paragraph 2.1.4.2 of CLP Regulation, if the exothermic decomposition energy of organic materials is less than 800 J/g, a UN gap test is not required, neither according to Series 1 Type (a) nor according to Series 2 Type (a). Since mefentrifluconazole has an exothermic decomposition energy of 580 J/g, waiving this test was appropriate.

Mefentrifluconazole was negative in a Koenen test but not in a time/ pressure test. In summary, the substance was tested in one out of two of the required tests: while it proved thermal stability, the data regarding ignition under confinement are lacking.

If a time/pressure test would have shown negative conclusions, then mefentrifluconazole would have been classified as *not explosive*. If the decision logic is followed further, then another question has to be answered: "Is the substance / mixture too insensitive for acceptance into this Class?" The response is give following the results obtained in Test Series 2. This battery of assays comprises the same tests as the Series 1 with a slight modification: the UN Gap test is performed with a defined gap of 50 mm. However, this test is waived as shown before.

Therefore, the time/pressure test appears to be crucial in the decision logic regarding the choice of *no classification* for explosion properties: if it is negative the substance is not classified but if it is positive then the line of decision has to be continued. This test determines if the ignition under confinement leads to a deflagration with explosive violence at pressures which can be attained with substance in normal commercial packages. However, it should be noted that, even though failure to ignite by flame does not necessarily indicate the lack of explosive properties, mefentrifluconazole could not be ignited by flame or melted in an open atmosphere during the test of flammability under *EU A.10 Flammability.* Also, when subjected to intense heat under high confinement (Koenen test), mefentrifluconazole did not show any explosion properties. These two tests corroborated with the calorimetric profile indicate a low energy substance.

The EU *A.14. Explosive properties* method has been used in classifications over time and comprises three basic tests for investigating the behaviour to ignition, friction and shock. The last two properties are also investigated in the actual scheme for classification incorporated in Test Series 3:

Type 3 (a): a falling weight test to determine sensitiveness to impact (BAM Fallhammer);

Type 3 (b): a friction; or impacted friction test to determine sensitiveness to friction (BAM friction apparatus);

Type 3 (c): an elevated temperature test to determine thermal stability (thermal stability test at 75 °C); and

Type 3 (d): an ignition test to determine the response of a substance or mixture to fire (small scale burning test).

According to the CLP Guidance (paragraph 2.1.4.5.1, acceptance procedure), it is recommended to carry out Test Series 3 before Test Series 1 and 2 for safety reasons due to the small sample amount needed. It is also recommended to carry out Test Series 3 even if negative results have been obtained in Test Series 1 and/or 2 because only Test Series 3 gives information about the thermal stability and the sensitivity to mechanical stimuli (impact and friction).

Mefentrifluconazole successfully passed the 3(a) and 3(b) tests but was not subjected to the remaining two.

The elevated temperature test is used to assess the thermal stability for prolonged (48 h) exposure to heat. If the temperature of the test substance rises more than 1.5 °C the substance indicates self-heating properties. Still, mefentrifluconazole was not classified for self-heating properties due to its low melting point.

The small scale burning test is used to assess the response to fire: the substance fails to ignite, ignites and burns or explodes. The test is performed in open atmosphere as in the *EU A.10* protocol in which mefentrifluconazole did not ignite and melted.

Conclusions on explosive properties

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.

When assessed using the decision logic from the CLP regulation, the screening procedure raises attention regarding the explosive properties due to the chemical structure and the energy release in the calorimetric profile. However, these findings cannot unequivocally indicate the need for testing; consequently, the decision between no classification based on waiving the tests and continuing with testing is a borderline case.

Following the decision logic, RAC agrees that the substance should be assessed taking the path for substances not manufactured for explosive purposes. Since mefentrifluconazole is also not a candidate for ammonium nitrate emulsion or gel, in order to validate the no classification or further proceed with the acceptance procedure, the results of a three test battery should be assessed. The first test can be undoubtedly waived based on CLP Criteria. The reason is the low decomposition energy which is a general characteristic of mefentrifluconazole. The second test, heating under confinement, proved negative but the third test, igniting under confinement, was not performed. Consequently, one of the three necessary pieces of information is missing. However, it should be noted that the physical hazard profile indicates that mefentrifluconazole is not a reactive substance: neither unstable properties (pyrophoric, flammable gases emissions in contact with water) nor reactivity (flammability, self-heating) were shown. This low reactivity is further shown in terms of explosive properties. On the other hand, it does not meet the required testing criteria for propagation of a detonation due to its low energy release profile. The inability of triazole compounds to initiate or propagate an explosive event is supported by the actual practice: similar compounds in usage are not known to exhibit explosive properties.

Mefentrifluconazole proved negative in three important tests of initiation of an explosive reaction: heat, shock and friction. Therefore, no classification is proposed.

Oxidising solids

According to the CLP Regulation and method EU A.17, oxidising properties (solids) a substance is considered as oxidising "*if in the full test, the maximum burning rate of the mixtures tested is higher than or equal to the maximum burning rate of the reference mixture of cellulose and barium nitrate."* Therefore, according to this method mefentrifluconazole did not show oxidising properties.

However, the *EU A.17* 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 the *Recommendations on the transport of Dangerous Goods, Manual of test and criteria, edited by the United Nations (UN RTDG, Manual of Tests and Criteria)*, namely *UN O.1 Test for oxidising solids* and *UN O.3 Gravimetric test for oxidising solids*.

All the above-mentioned methods are based on the same principle: the test substance is mixed with a combustible substance (dry fibrous cellulose), followed by the comparison of the burning intensity/rate against a mixture of a reference substance and cellulose. In the EU A.17 method, the comparison is made against a single cellulose mixture/ratio. Consequently, the substance can be categorised as having oxidising properties or not but no sub-categories can be assigned. In the UN methods, the comparison is made against different ratios of cellulose mixtures. Therefore, hazard categories can be assigned as specified by the CLP Regulation. Moreover, the reference substance is different, although alternatives can be accepted.

Mefentrifluconazole was not classified under the DSD, but was tested using a method compliant with this directive repealed by CLP Regulation. Previous versions of the CLP Guidance such as *ECHA-09-G-02* published on 25/08/2009 or *Version 4.1* – June 2015 specify that: "*In general, solids that were classified as oxidising according to DSD/DPD will also meet the criteria for classification as oxidising solids according to the CLP. However, unless the proper Category can be assigned on the basis of other available data (e.g. through the transport classification), re-testing will be necessary."* In the last version of the CLP Guidance (Version 5.0 – July 2017), a correspondence between the transport classification and the CLP is given. In the case of mefentrifluconazole neither a previous classification was made nor is a transportation classification provided by the DS.

The common and decisive step in the decision logic of the CLP versions is the first one: if the mixture of the test substance with cellulose does not ignite or burn then the substance does not meet the criteria for classification. In the case of mefentrifluconazole the mixture ignited and burned under the conditions of the EU A.17 method.

In addition, waiving the tests can be done if "*For organic substances or mixtures the classification procedure for this hazard class need not be applied if: …b. the substance or mixture contains oxygen, fluorine or chlorine and these elements are chemically bonded only to carbon or hydrogen*." The chemical structure of mefentrifluconazole fulfils this condition.

When referring to the experience in the handling and use of the substance, there is no report or mention that similar triazole compounds would present oxidising properties.

In summary, according to CLP Guidance, section 2.14.4.1.1. *Screening procedures and waiving of testing,* mefentrifluconazole does not need not to be classified. 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. Consequently, RAC agrees that there is sufficient evidence to warrant **no classification** of mefentrifluconazole for Oxidising properties under the CLP regulation.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

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

The absorption, distribution, excretion and metabolism of mefentrifluconazole in mammals were investigated with the active substance radiolabelled in either the chlorophenyl ring (C-label), the trifluoromethylphenyl ring (TFMP-label) or the triazole moiety (T-label).

The plasma kinetics of mefentrifluconazole in rats and mice demonstrated high absorption following oral administration, indicated potential enterohepatic recirculation of the triazole moiety, and showed fast excretion and a more-or-less linear correlation of the internal exposure to the oral dose. The biliary excretion data confirmed that oral absorption in rats was approximately 85 % following single low-dose administration; the same oral absorption value in humans will be assumed. The biliary excretion experiments also confirmed that excretion was fast, more or less complete and occurred to a major extent within three days after oral dosing in rats, predominantly by the faecal route. There was no evidence of accumulation. The excretion patterns for males and females were similar with the C- and TMFP-labels, but were somewhat different for the T-label; in this case, urinary excretion was higher for male than for female animals, especially in the low dose group.

In addition to the information provided by the plasma kinetics' data, evidence of enterohepatic recirculation of the triazole moiety was provided by the biliary-excretion investigations. The distribution experiments demonstrated that mefentrifluconazole was rapidly and widely distributed in rats after a single oral administration. The active substance was extensively and rapidly metabolised after single and multiple oral doses, with 68 metabolites having been identified; the parent compound was the main source of radioactivity recovered from faeces and to some extent the liver, but was not present in bile or urine.

In a comparative *in vitro* metabolism study, one metabolite was detected following incubation with human hepatocytes, which was also detected in rat hepatocyte samples; hence, the study did not detect any unique human metabolite.

In a guideline-compliant *in vitro* study with human skin, the dermal absorption of mefentrifluconazole was estimated to be 4 % from a concentrate formulation (100 mg/ml) and 8 % from a 1:200 dilution (0.5 mg/ml).

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

The acute toxicity of mefentrifluconazole has been investigated by the oral, dermal and inhalation routes. *In vitro* and *in vivo* irritation studies are available, whilst skin sensitisation has been investigated in a guinea pig maximisation test.

10.1 Acute toxicity - oral route

The acute-toxic-class method has been used to investigate the acute oral toxicity of mefentrifluconazole.

Table 12: Summary table of acute oral toxicity studies with mefentrifluconazole

Method, guideline, deviations if any	Species, strain, sex, no/group	Dose levels	Value LD_{50}	Remarks
OECD 423 (2001) (acute toxic class method) GLP Report CA 5.2.1/1 [redacted], 2013c	Rat, Wistar, 3 females / group (2 groups)	2000 mg/kg bw suspended in corn oil Purity 98.8%	> 2000 mg/kg bw	No deaths. Clinical signs included cowering position, impaired general state and piloerection between 2 and 5 hours after administration. No adverse macroscopic necropsy findings

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

In an acute oral toxicity study performed in accordance with the acute toxic class method (OECD 423), 2000 mg/kg mefentrifluconazole was administered to three fasted female rats. As no deaths occurred in this group, the result was confirmed in three additional animals at the same dose level.

None of the animals died. The observed clinical signs were indicative of general toxicity and did not give any indication of specific target-organ toxicity; moreover, they had resolved within a few hours of the dose being administered, and gross pathology did not reveal any adverse findings. The mean body weight of the animals increased throughout the study period within the normal range.

10.1.2 Comparison with the CLP criteria

In accordance with the CLP criteria, substances are classified for acute oral toxicity if the LD50 value is ≤ 2000 mg/kg. Since in the available study the LD50 value of mefentrifluconazole was > 2000 mg/kg, it is concluded that the substance does not meet the criteria for classification.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Not classified (conclusive but not sufficient for classification).

10.2 Acute toxicity - dermal route

One acute dermal toxicity study is available, which was conducted in rats.

Table 13: Summary table of acute dermal toxicity studies with mefentrifluconazole

Method, guideline, deviations if any	Species, strain, sex, no/group	Dose levels duration of exposure	Value LD_{50}	Remarks
OECD 402 (1987) GLP Report CA 5.2.2/1 [redacted], 2013b	Rat, Wistar, 5/sex Observation period: 14 days	5000 mg/kg suspended in corn oil, applied for 24 hours Purity 98.8%	> 5000 mg/kg	No deaths. No signs of systemic toxicity or skin effects. No adverse macroscopic necropsy findings.

10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

In an acute dermal toxicity study, rats were exposed to a single limit dose of 5000 mg/kg mefentrifluconazole for 24 hours under a semi-occlusive dressing. The application area comprised at least 10 % of the total body surface area. At the end of the 24-hour exposure period, the dressing was removed and the application site was rinsed with warm water. Skin effects were monitored 30-60 minutes after removal of the dressing, weekly thereafter and on the last day of observation. There were no deaths, signs of systemic toxicity, local skin effects or adverse macroscopic findings at necropsy. The mean body weights of the animals increased within the normal range throughout the study period.

10.2.2 Comparison with the CLP criteria

In accordance with the CLP criteria, substances are classified for acute dermal toxicity if the LD50 value is ≤ 2000 mg/kg. Since in the available study the LD50 value of mefentrifluconazole was > 5000 mg/kg, it is concluded that the substance does not meet the criteria for classification.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Not classified (conclusive but not sufficient for classification).

10.3 Acute toxicity - inhalation route

A limit test to investigate the acute toxicity of mefentrifluconazole by the inhalation route has been conducted in rats.

Table 14: Summary table of acute inhalation toxicity studies with mefentrifluconazole

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Value LC_{50}	Remarks
OECD 403 (2009) GLP Report CA 5.2.3/1 [redacted], 2014a	Rat, Wistar, 5/sex Head/nose exposure Observation period: 14 days	5.3 mg/l (analytical concentration) as a dust aerosol for 4 hours Purity 98.8% Mass median aerodynamic diameters (MMADs) of 3.8 µm	> 5.3 mg/l	No deaths. Clinical signs included laboured breathing, abdominal respiration, respiratory sounds, encrusted eyes, red & colourless discharge and/or red crusts of the nose, poor general state, hunched posture, hyper-excitability, no defecation, piloerection and substance-contaminated fur; observed from 2 hours to 11 days after exposure. No adverse macroscopic necropsy findings.

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

In an acute inhalation study, rats were exposed in a head/nose-only system for four hours to a limit concentration of 5.3 mg/l of mefentrifluconazole as a dust aerosol. There were no deaths. General indications of toxicity and respiratory effects that are commonly associated with the inhalation route of exposure were observed between two hours and 11 days after the exposure. No clinical symptoms were recorded from day 12 onwards. Body weights increased as expected from day three onwards and there were no adverse macroscopic findings upon necropsy. It was 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 influence the test results because of the relatively short exposure time.

10.3.2 Comparison with the CLP criteria

In accordance with the CLP criteria, a substance is classified for acute inhalation toxicity if the 4-h LC50 value is less than 5 mg/L. Since in the available study the LC50 value of mefentrifluconazole was > 5.3 mg/L, it is concluded that the substance does not meet the criteria for classification.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Not classified (conclusive but not sufficient for classification).

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.

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 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 adopted 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.

10.4 Skin corrosion/irritation

Information on the skin irritation potential of mefentrifluconazole is available from a non-GLP *in vitro* study and a GLP-compliant rabbit study.

Table 15: Summary table of studies to investigate the skin corrosion/irritation potential of mefentrifluconazole

Method, guideline, deviations if any	Test system	Dose levels duration of exposure	Results
EpiDerm skin corrosion / irritation test OECD 431 Not GLP Report CA 5.2.4/1 Remmele, 2012a	Human reconstituted epidermis model exposed for 3 minutes & 1 hour (corrosion test) or 1 hour with 42 hours post-incubation (irritation test)	25 µl bulk volume (approx. 11 mg), minimally moistened with water Purity 97.7 %	Tissue viability values were comparable to the negative control (100-102 % of the negative control values) for all experiments. The positive control substance (5 % SDS for irritation test) resulted in reduced tissue viability (3 % of the negative control) Not a skin irritant under the conditions of the study.
Acute dermal irritation / corrosion in rabbits OECD 404 (2002) GLP Report CA 5.2.4/2 [redacted] 2013a	Rabbit, New Zealand White, 3 females (step-wise procedure)	0.5 g minimally moistened with water applied to intact skin for 4 hours under semi-occlusive dressing. Purity 98.8 %	Mean scores (averaged over 24, 48 & 72 hours) for each animal: 0, 0, 0 for erythema 0, 0, 0 for oedema Not a skin irritant.

10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

An *in vitro* study with the EpiDerm™ human skin model was performed as a pre-test. The cell viability, as measured by dehydrogenase conversion of the yellow, water-soluble MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide), into a blue formazan salt, was comparable between the mefentrifluconazole-exposed samples and the negative controls. The reductions in cell viability with the positive control substances demonstrated the sensitivity of the system. The test material did not show an irritant potential under the conditions of this study.

The *in vivo* study was conducted in a step-wise procedure; an initial animal that showed there were no severe skin lesions was subsequently supplemented with two additional rabbits. Slight erythema (grade 1) was observed in one of the three treated animals immediately after removal of the patch; this was reversible within one hour. No other cutaneous reactions were observed during the study. Mean scores over 24, 48 and 72 hours for each animal were 0.0, 0.0 and 0.0 for both erythema and oedema.

10.4.2 Comparison with the CLP criteria

A substance is classified as a skin irritant category 2 if any of the following criteria are met:

(1) mean value of ≥ 2.3 - ≤ 4.0 for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions; or

(2) inflammation that persists to the end of the observation period, normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling; or

(3) in some cases where there is pronounced variability of response among animals, with very definite positive effects related to chemical exposure in a single animal but less than the criteria above.

Since the average scores for each animal were 0.0, 0.0 and 0.0 for erythema and 0.0, 0.0 and 0.0 for oedema in the rabbit study, with supportive information provided by the *in vitro* test, none of these criteria was met.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Not classified (conclusive but not sufficient for classification).

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.

10.5 Serious eye damage/eye irritation

The eye irritation potential of mefentrifluconazole has been investigated in two non-GLP *in vitro* tests and a GLP-compliant test in rabbits.

Table 16: Summary table of studies to investigate the eye irritation potential of mefentrifluconazole

Method, guideline, deviations if any	**Test system**	**Dose levels duration of exposure**	**Results**
EpiOcular eye irritation test Not guideline or GLP Report CA 5.2.5/1 Remmele, 2012b	2 EpiOcular tissue samples	50 µl bulk volume (approx. 15 mg) minimally moistened with water, exposed for 90 minutes followed by 18 hours' post-incubation period Purity 97.7 %	Tissue viability values were 81 % of the negative control. Positive control (methyl acetate) resulted in tissue viability of 20 % of negative control value. No eye irritation potential.
Bovine corneal opacity & permeability test (BCOP) OECD 437 Not GLP Report CA 5.2.5/2 Remmele 2012c	Three bovine corneas	750 µl of 20 % solution in water, exposed for 4 hours Purity 97.7 %	Mean *in vitro* irritancy scores (IVIS): BAS 750 F = -0.4 ± 2.1 Negative control = 5.5 ± 2.5 Positive control (20% imidazole) = 118.3 ± 3.6 Histopathology did not reveal findings that indicated eye damage. No serious eye damage potential.
Acute eye irritation in rabbits OECD 405 (2002) GLP Report CA 5.2.5/3 [redacted], 2013a	Rabbit, New Zealand White, 3 (step-wise procedure)	0.1 ml bulk volume (approx. 38 mg) applied in one eye for 24 hours, followed by rinsing with tap water. Purity 98.8 %	Mean scores (averaged over 24, 48 & 72 hours) for each animal: 0, 0, 0 for corneal opacity 0, 0, 0 for iris lesions 0.3, 0.3, 0.7 for redness of the conjunctiva 0, 0, 0 for conjunctival chemosis All reactions reversible within 72 hours after application.

10.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

Two *in vitro* pre-tests were performed to evaluate the potential for corrosion / severe eye damage and eye irritation before an *in vivo* test was undertaken.

In an EpiOcular™ test, tissue destruction was determined by measurement of the metabolic activity of the tissue after exposure/post-incubation with a colorimetric test (reduction of MTT to its formazan salt). In the system employed, a substance was considered to be irritant if the mean relative tissue viability with the test material was less than or equal to 50 % of the negative control value. On this basis, the mean relative tissue viability obtained with mefentrifluconazole (81 %) was concluded to indicate that there was not an eye irritation potential.

In the BCOP assay, corneal opacity was measured quantitatively as the amount of light transmission through the cornea. Permeability was measured quantitatively as the amount of sodium fluorescein dye that passed across the full thickness of the cornea. Both measurements were used to calculate an *in vitro* irritancy score (IVIS) of the test substance (= mean opacity value + (15 x mean OD490)), which is used for the prediction of serious eye damage. In addition, histological evaluation was performed. A substance was considered to represent a risk of serious damage to the eyes if the IVIS was > 55. On this basis, it was concluded that mefentrifluconazole did not represent a risk of serious eye damage (IVIS = -0.4).

The follow-up *in vivo* study was conducted in a step-wise procedure: an initial animal was used to establish a potential for severe lesions, which was subsequently supplemented with two additional animals. In addition to the readings at 1, 24, 48 and 72 hours, an additional examination was performed at 24 and 48 hours with the installation of fluorescein. There were no signs of gross toxicity, adverse clinical signs or abnormal behaviour following administration of the test substance. Slight conjunctival redness (grade 1) was noted in all three animals at hours 1 and 24 after application and persisted in one animal up to hour 48. Slight conjunctival chemosis (grade 1) was noted in one out of three animals 1 hour after application. Slight discharge (grade 1) was noted in two out of three animals 1 hour after application. Additional findings, for example injected scleral vessels in a circumscribed area, were noted in all animals at hour 1 and persisted in two animals up to 24 hours. No corneal lesions were detectable even after instillation of fluorescein performed 24 and 48 hours after application. The mean scores calculated for each animal over 24, 48 and 72 hours were 0.0, 0.0 and 0.0 for corneal opacity, iris lesions and conjunctival chemosis and 0.3, 0.3 and 0.7 for redness of the conjunctiva. The ocular reactions were fully reversible within 72 hours after application.

10.5.2 Comparison with the CLP criteria

In accordance with CLP, a substance is classified as an eye irritant category 2 if, when applied to the eye of an animal, the substance produces in at least 2 of 3 tested animals a positive response of:

- corneal opacity ≥ 1 and/or
- iritis ≥ 1, and/or
- conjunctival redness ≥ 2 and/or
- conjunctival oedema (chemosis) ≥ 2,

calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material, and which fully reverses within an observation period of 21 days.

In the rabbit test with mefentrifluconazole, the individual and overall mean eye irritation scores (24 to 72 hours) were 0.0 for corneal opacity, iris lesions, and for conjunctival chemosis. The mean scores for conjunctival redness for each animal were 0.3, 0.3 and 0.7 and were thus less than those that trigger classification. Furthermore, the reactions seen were fully reversible within 72 hours. Additional, supportive

information was provided by the *in vitro* tests, in which mefentrifluconazole showed neither a serious eye damage potential nor an eye irritation potential.

10.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

Not classified (conclusive but not sufficient for classification).

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.

10.6 Respiratory sensitisation

No studies available.

10.6.1 Conclusion on classification and labelling for respiratory sensitisation

Because of the lack of data, a definitive conclusion on respiratory sensitisation cannot be made.

10.7 Skin sensitisation

The skin sensitisation potential of mefentrifluconazole has been investigated in a guinea-pig maximisation test.

Table 17: Summary table of studies to investigate skin sensitisation potential

<table>
<tr><th>Method, guideline</th><th>Species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th></tr>
<tr>
<td>Guinea-pig maximisation test (GPMT)
OECD 406 (1992)
GLP
Report CA 5.2.6/1
[redacted] 2013a</td>
<td>Guinea pigs, Dunkin-Hartley, females, 5 in control group & 10 in test group</td>
<td>Intra-dermal induction: 5% in paraffin oil or 5% in Freund’s complete adjuvant/0.9% aqueous NaCl
Topical induction: 60% in paraffin oil
Challenge: 50% in paraffin oil
Purity 98.8%</td>
<td>Responses after challenge:
<table>
<tr><th></th><th>24h</th><th>48h</th><th>24 or 48h</th></tr>
<tr><td>Control</td><td>0/5</td><td>0/5</td><td>0/5</td></tr>
<tr><td>Test</td><td>2/10</td><td>6/10</td><td>6/10</td></tr>
</table>
All positive responses consisted of grade 1 erythema.
Positive control (separate study) α-hexylcinnamaldehyde = 7/10</td>
</tr>
</table>

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

The induction and challenge concentrations of mefentrifluconazole employed in the available GPMT were determined from a series of pre-tests. In the pre-tests, paraffin oil showed satisfactory results as a vehicle for intra-dermal injection, whereas other commonly-used vehicles (1% aqueous carboxy methylcellulose, polyethylene glycol 400) were demonstrated to be unsuitable. The maximum injectable concentration of mefentrifluconazole was 5% (w/w) suspension, and hence was used for the intra-dermal induction. Two animals pre-treated with the adjuvant were used to determine the minimal irritating concentration of mefentrifluconazole for the topical induction and the maximum non-irritating concentration for the challenge application. Based on the results of this pre-test, a concentration of 60% (w/w) was selected for topical induction and a concentration of 50% (w/w) for topical challenge.

In the main test, intra-dermal injection of 5% (w/w) substance in the adjuvant mixture caused skin irritation (grade 1 to 2) and necrosis in the test and control groups. The sites of injections with only mefentrifluconazole or vehicle showed none or a slight erythema but without necrosis. Necrosis was observed during the topical induction phase at the sites of adjuvant administration in both groups, but not at the injection sites without the use of adjuvant, nor did these sites show discernible erythema.

None of the control animals responded with skin reactions during the challenge. In the test group, 6 of 10 animals showed skin reactions in the form of discrete or patchy erythema (grade 1); two of these animals additionally presented with papules 24 and/or 48 hours after the challenge. The challenge treatment with the vehicle alone did not cause skin reactions in any animals of the test group. As 60 % of animals gave a positive response in this adjuvant test, it is concluded that a skin sensitisation potential was demonstrated under the conditions of the study.

10.7.2 Comparison with the CLP criteria

The sub-categorisation of skin sensitisers on the basis of a GPMT is illustrated in the table below. In the case of the GPMT study with mefentrifluconazole, 60 % of the test animals responded to a 5 % intradermal induction dose and thus allow one to conclude that classification in at least sub-category 1B is warranted; however, since an intra-dermal induction concentration below 0.1 % was not tested, it is not possible to exclude a classification in sub-category 1A. In this situation, the guidance on the application of the CLP criteria[1] 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 enough sensitiser to

[1] Guidance on the application of the CLP criteria, version 4.0, November 2013, ECHA.

merit classification in category 1A, based upon the intra-dermal induction concentration that gave a response in 60 % of animals, the dossier submitter proposes classification in category 1.

Table 18: Potency on basis of the Guinea Pig Maximisation Test

Concentration for intradermal induction (% w/v)	Incidence sensitised guinea pigs (%)	Potency	Predicted subcategory
≤ 0.1	≥ 60	Extreme	1A
≤ 0.1	≥ 30 - < 60	Strong	1A
>0.1 - ≤ 1.0	≥ 60	Strong	1A
>0.1 - ≤ 1.0	≥ 30 - < 60	Moderate	1B
> 1.0	≥ 30	Moderate	1B

10.7.3 Conclusion on classification and labelling for skin sensitisation

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

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

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.**

10.8 Germ cell mutagenicity

The genotoxic potential of mefentrifluconazole has been investigated in a series of *in vitro* studies and an *in vivo* micronucleus test.

In vitro

The potential of mefentrifluconazole to induce gene mutations in bacterial cells, gene mutation/clastogenicity in mammalian cells and clastogenicity/aneuploidy in mammalian cells has been investigated in *in vitro* studies.

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

Method, guideline, deviations if any	Test system (Organism, strain)	Test substance	Conc. tested (range)	Results		Remarks (information on cytotoxicity)
				–S9	+S9	
Gene mutation assay in bacteria (Ames) Plate incorporation & pre-incubation OECD 471, GLP	*S.typhimurium* strains TA 1535, TA 1537, TA 98, TA 100; *E.coli* strain WP2 uvrA	Purity 98.6% Woitkowiak, 2014a	1 - 5000 µg/plate	neg	neg	Plate incorporation: toxicity at ≥ 333 µg/plate Pre-incubation: toxicity at ≥ 33 µg/plate
		Purity 97.9% Woitkowiak, 2015a	3.3 - 5000 µg/plate	neg	neg	Toxicity at ≥ 100 µg/plate (±S9) Precipitation at ≥ 333 µg/plate
Gene mutation assay in mammalian cells OECD 476, GLP	Mouse lymphoma L5178Y cells TK +/- locus	Purity 98.8% Wollny, 2015a	3.75 - 60 µg/mL	neg	neg	Toxicity at ≥ 30 µg/mL (±S9, 24 h)
		Purity 97.9% Wollny, 2015b	3.1 – 62.5 µg/mL	neg	neg	–S9: toxicity at ≥ 12.5 µg/mL (24 h) +S9: toxicity at ≥ 37.5 µg/mL (4 h)
In vitro micronucleus assay OECD 487, GLP	V79 Chinese Hamster lung fibroblasts	Purity 98.8% Schulz & Landsiedel, 2014a	0.39 - 50 µg/mL	neg	neg	–S9: toxicity at ≥ 3.13 µg/mL (24 h) +S9: toxicity at 50 µg/mL (4 h)
	Human lymphocytes	Purity 97.9% Sokolowski, 2015a	2.0 - 8.2 µg/mL	neg	neg	Toxicity at 8.2 µg/mL (±S9)

Six well-conducted, reliable *in vitro* studies have been conducted to investigate the *in vitro* genotoxic potential of mefentrifluconazole in bacterial and mammalian cells. There was no indication of a genotoxic response to mefentrifluconazole in any of the assays. Cytotoxicity, which was evident in each test, indicated that adequate test concentrations had been used.

In vivo

A micronucleus test has been conducted to investigate the potential of mefentrifluconazole to induce chromosomal damage in mice.

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

Method, guideline, deviations if any	Species, strain, sex, No/group	Test substance	Dose levels and sampling times	Results
Micronucleus test OECD 474, GLP [redacted]., 2014a	Mouse, NMRI, males, 5/group	Purity 98.8% Vehicle: DMSO/corn oil (2:3 ratio)	0, 375, 750, 1500 mg/kg bw, single oral (gavage) dose Bone marrow sampled at 24 and 48 hours	Negative 2000 polychromatic erythrocytes evaluated per animal. Clinical signs of systemic toxicity noted in all animals in all dose groups. PCE/NCE ratio decreased with 1500 mg/kg mefentrifluconazole at 48 hours (1.47 vs 2.26 in negative controls) Mefentrifluconazole demonstrated in blood samples.

In a micronucleus test in male NMRI mice, mefentrifluconazole was administered in a single oral dose of 375, 750 and 1500 mg/kg in a volume of 20 ml/kg body weight. The vehicle served as the negative control and CPA and vincristine as the positive controls. The animals were sacrificed 24 or 48 (additional high-dose group) hours after the administration, with the bone marrow of the two femora being prepared from each animal. For each animal, 2000 polychromatic erythrocytes were evaluated for micronuclei, therefore 10 000 were scored per test group. The normocytes occurring per 2000 polychromatic erythrocytes were also recorded. Blood samples taken immediately after sacrifice were analysed to verify the bioavailability of the test substance.

Administration of mefentrifluconazole did not lead to any biologically relevant increase in the number of polychromatic erythrocytes that contained micronuclei. The rate of micronuclei was close to the concurrent negative control and within the range of the historical control data. The positive-control for clastogenicity, CPA, led to the expected increase in the rate of polychromatic erythrocytes that contained exclusively small micronuclei, whilst vincristine, which is a spindle poison, produced a statistically significant increase in micronuclei, approximately 10 % of which were attributable to large micronuclei.

The bioavailability of the test substance in blood after oral administration was confirmed in plasma samples from all test animals and also in a biokinetics' study conducted in mice (section 9.1). Since the bone-marrow is well perfused, it is expected to be exposed to mefentrifluconazole or its metabolites. Furthermore, a slight inhibition of erythropoiesis (decreased PCE/NCE ratio) in the top-dose group at the 48-hour sacrifice indicated bone-marrow toxicity. Clinical signs, which were observed in all dose groups, included piloerection, hunched posture, reduced general condition, lacrimation and irregular respiration. Overall, the dossier submitter concludes that a valid negative result was obtained in this study.

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

Mefentrifluconazole has been tested for its potential genotoxic properties in a battery of *in vitro* assays and one *in vivo* test.

The exposure of *Salmonella typhimurium* and *Escherichia coli* tester strains to mefentrifluconazole up to and including the limit concentration of 5000 µg/plate did not produce an increased number of reversions, either with or without metabolic activation. Two *in vitro* assays for gene mutations (thymidine kinase locus) in mouse lymphoma cells with and without S9-mix were negative, even when tested up to precipitating concentrations

of mefentrifluconazole. Likewise, the substance did not induce any evidence of clastogenic or aneugenic effects in micronucleus tests in V79 cells and human lymphocytes with or without S9-mix.

The genotoxicity of mefentrifluconazole was tested *in vivo* in a bone marrow micronucleus assay in NMRI mice. The test did not reveal an increased frequency of micronucleated polychromatic erythrocytes following single oral doses of up to 1500 mg/kg bw. Taking into account the plasma-kinetics of mefentrifluconazole after oral administration, the clinical signs of systemic toxicity and the reduced PCE/NCE ratio, which is indicative of an effect on erythropoiesis and hence bone-marrow toxicity, the negative result is valid.

Based on the available data set, mefentrifluconazole is concluded not to be a somatic-cell mutagen. Its potential to be a germ-cell mutagen has not been investigated.

10.8.2 Comparison with the CLP criteria

There was no evidence of a genotoxic potential in six *in vitro* assays and one *in vivo* guideline- and GLP-compliant assay.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Not classified (conclusive but not sufficient for classification).

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.

10.9 Carcinogenicity

The carcinogenic potential of mefentrifluconazole in animals has been investigated in rats and mice.

Table 21: Summary table of animal studies on carcinogenicity

<table>
<tr><th>Method
Guideline,
Deviation(s) from the guideline (if any)</th><th>Species
Strain
Sex
no/group</th><th>Dose levels
duration of exposure</th><th>Results</th></tr>
<tr><td>Two-year combined chronic toxicity / carcinogenicity
Oral (dietary)
OECD 453
GLP
Purity 98.8 %
[redacted]., 2016b</td><td>Rat, Wistar,
10/sex/dose (1-yr)
50/sex/dose (2-yr)</td><td>0, 100, 600, 3600 ppm
Equivalent intake at 24-months:
Males: 0, 4, 25, 163mg/kg bw/d
Females: 0, 6, 38, 302mg/kg bw/d</td><td><u>Chronic phase – 12 months</u>
No deaths in any dose group. No overt clinical signs of toxicity.
<u>5 / 7 mg/kg bw/d:</u>
No adverse effects
<u>31 / 41 mg/kg bw/d:</u>
Haematology: ↓ activated partial thromboplastin time in males (statistically significant but < 5 % change at 12 months)
Altered clinical chemistry parameters in males (↑ ALP & urea)
<u>191 / 300 mg/kg bw/d:</u>
↓ mean bw (final bw -8.3%** in males, -13.8%** in females) and bwg (overall -12%** in males, -27.1%** in females)
Haematology: ↓ activated partial thromboplastin time in males & females (statistically significant but < 5 % change at 12 months), ↓ platelet counts in males (statistically significant but < 5 % change at 12 months)
Altered clinical chemistry parameters in males & females (↑ ALP, cholesterol, glucose, urea; ↓ total protein, albumin, creatinine)
Increased liver weight (9-22 %, males & females) & centrilobular hypertrophy (minimal / slight: 6/10 males, 5/5 females)
<u>Carcinogenicity phase – 24 months</u>
Survival to day 728 (24 months):
<table>
<tr><th rowspan="2">Dose level (ppm)</th><th colspan="2">Survival (%)</th></tr>
<tr><th>Males</th><th>Females</th></tr>
<tr><td>0</td><td>100</td><td>76 (38/50)</td></tr>
<tr><td>100</td><td>76 (38/50)</td><td>80 (40/50)</td></tr>
<tr><td>600</td><td>78 (39/50)</td><td>82 (41/50)</td></tr>
<tr><td>3600</td><td>94 (47/50)</td><td>90 (45/50)</td></tr>
</table>
<u>Non-neoplastic effects</u>
No overt clinical signs of toxicity in any group.
<u>4 / 6 mg/kg bw/d:</u>
No adverse effects
<u>25 / 38 mg/kg bw/d:</u></td></tr>
</table>

<table>
<tr><th>Method
Guideline,
Deviation(s) from the guideline (if any)</th><th>Species
Strain
Sex
no/group</th><th>Dose levels
duration of exposure</th><th>Results</th></tr>
<tr><td></td><td></td><td></td><td>↑ relative liver wt in females (16 %)
<u>163 / 302 mg/kg bw/d:</u>
↓ mean bw (final bw -11.6%** in males & -21.9%** in females) and bwg (overall bwg -15.7%** in males, -35.1%** in females)
↑ relative liver wt (7 & 23 %, males & females). Minimal hepatocellular hypertrophy (15**/50 males, 7**/50 females)
<u>Neoplastic findings</u>
Slight increases in incidences of malignant lymphoma in males (0, 4 %, 4 %, 6 % at 0, 5, 29, 185 mg/kg bw/d) & uterine adenocarcinoma in females (2 %, 18 %, 8 %, 10 % at 0, 6, 41, 312 mg/kg bw/d)
Historical control data (24-month data from 12 studies in Wistar rats, started Jan-2003 to Feb-2013):
Male - malignant lymphoma: mean: 2.5 %; min.: 0 %; max. 6 %
Female - uterus, adenocarcinoma, endometrial: mean: 16.2 %; min.: 2 %; max: 30%</td></tr>
<tr><td>18-month carcinogenicity
Oral (dietary)
OECD 451
GLP
[redacted]
2015b</td><td>Mouse, C57BL/6JRj
50/sex/dose</td><td>Males: 0, 20, 50, 200 ppm
Equivalent to 0, 3.5, 9.1, 36 mg/kg bw/d
Females: 0, 20, 50, 250 ppm
Equivalent to 0, 4.9, 12.6, 61.5 mg/kg bw/d</td><td>Survival to termination:
<table>
<tr><th rowspan="2">Dose level
(mg/kg bw/d)</th><th colspan="2">Survival (%)</th></tr>
<tr><th>Males</th><th>Females[a]</th></tr>
<tr><td>0</td><td>98 (49/50)</td><td>94 (46/49)</td></tr>
<tr><td>3.5 / 4.9</td><td>96 (48/50)</td><td>96 (46/48)</td></tr>
<tr><td>9.1 / 12.6</td><td>100 (0/50)</td><td>95 (41/43)</td></tr>
<tr><td>36 / 61.5</td><td>100 (0/50)</td><td>89 (42/47)</td></tr>
</table>
No treatment-related overt clinical signs of toxicity.
<u>Non-neoplastic effects</u>
<u>3.5 / 4.9 mg/kg bw/d:</u>
No adverse effects
<u>9.1 / 12.6 mg/kg bw/d:</u>
Relative liver weight ↑ (18 %) with hepatocellular fatty change in males (change in severity score from 2.0 in controls to 2.9 in exposed group)
<u>36 / 61.5 mg/kg bw/d:</u>
↓ body wt & bwg (bwg up to -14 % in males, -33 % females)
Relative liver weight ↑ by 42 % males & 57 % females
Increased incidence & severity of fatty change and signs of (pre)degeneration in liver cells (eosinophilic inclusions in males, single cell necrosis in females)
↑ thyroid follicular-cell hyperplasia (74 % males compared with 42 % of controls)
<u>Neoplastic findings</u>
Thyroid follicular cell adenoma: 0, 0, 0, 4 % of males, 2%, 0,</td></tr>
</table>

Method Guideline, Deviation(s) from the guideline (if any)	**Species Strain Sex no/group**	**Dose levels duration of exposure**	**Results**
			2%, 6 % of females at 0, 20, 50, 200 / 250 ppm. Liver hepatocellular adenoma: 2 %, 4 %, 6 %, 0 % of males at 0, 20, 50, 200 ppm. None in females. *Historical control data (test facility, four studies, 2013-2014):* Males: thyroid, follicular cell adenoma: mean: 1.5 %; min.: 0 %; max. 2 % Females: thyroid, follicular cell adenoma: mean: 2.6 %; min.: 0 %; max: 6 %

bw = body weight; bwg = body-weight gain; * = statistically significant, $p \leq 0.05$; ** = statistically significant, $p \leq 0.01$
[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.

Chronic / carcinogenicity study in rats

In a combined chronic / carcinogenicity study, mefentrifluconazole was administered via the diet to Wistar rats over a period of either 12 or 24 months at dietary concentrations of 0, 100, 600 and 3600 ppm. The concentrations corresponded to mean intakes of: 12 months – 5, 31, 191 mg/kg bw/d in males and 7, 41, 300 mg/kg bw/d in females; 24 months – 4, 25, 163 mg/kg bw/d in males and 6, 38, 302 mg/kg bw/d in females.

There were no deaths in any of the satellite groups in the chronic phase of the study (12 months' exposure). In the main (carcinogenicity) groups, there was not a treatment-related increase in deaths. Survival exceeded 75 % in all groups. There were no overt clinical signs of toxicity in any group in either the satellite or main cohorts. Body weights and body-weight gain were reduced in the high-dose male and female groups after both 12 and 24 months of exposure from day 7 and then throughout the duration of the study. There were no statistically significant changes in body weight and body-weight gain in the low- and mid-dose groups. Food consumption and water consumption were unaffected at all doses.

Blood was collected for haematology and clinical-chemistry-parameter investigations from the satellite animals (12 months) at the 3, 6 and 12-month time-points. Treatment-related changes in haematology parameters consisted of slight decreases in the activated partial thromboplastin time (PTT) in males at 31 mg/kg bw/d and both sexes at 191 / 300 mg/kg bw/d, and a decrease in platelet counts in males at 191 mg/kg bw/d (12 months).

Several treatment-related changes in clinical-chemistry parameters were recorded at each time-point. These comprised increases in ALP and decreases in ALT, glucose and total bilirubin in males and females. Additionally, urea was increased in males of the mid- and high-dose groups. Creatinine was statistically significantly decreased and cholesterol increased in females at 300 mg/kg bw/d, but only at the three-month time-point. In high-dose females, albumin and total-protein levels were statistically significantly reduced at three and six months. The changes in ALT levels were relatively small and were probably an indication of liver-enzyme induction as a result of an adaptive response rather than adversity, whilst lower glucose levels perhaps reflected increased energy consumption as a result of greater liver-cell metabolism. Likewise, the lower total bilirubin levels probably reflected an increased conjugation rate of bilirubin and a subsequent higher excretion via bile and thus, although treatment-related, were not adverse. Overall, the clinical chemistry investigations indicated that adaptive changes and some alterations to liver-cell metabolism resulted from exposure to mefentrifluconazole.

Organ weights were measured after 12 and 24 months of exposure to mefentrifluconazole. At each time point, the only treatment-related, specific change (i.e., not secondary to a decrease in terminal body weight) was in liver weight. The liver weight relative to body weight was statistically significantly increased in high-dose

males (by 9 %) and females (by 22 %) at 12 months and in females (by 16 %) from 38 mg/kg bw/d at 24 months. At the latter time-point, the relative liver weight was increased by 7 % in males and 23 % in females at 163 / 302 mg/kg bw/d mefentrifluconazole.

There were no treatment-related findings upon gross necropsy at the 12- or 24-month intervals. Histopathology at 12 months did not reveal any treatment-related neoplasms in males or females. A minimal or slight treatment-related hepatocellular centrilobular hypertrophy was observed in the high-dose group in 6 / 10 males and 5 / 10 females. Histopathology at 24 months revealed statistically-significant increases in the incidences of non-neoplastic changes in the liver. Given the increases in relative weights, the findings in the liver (minimal centrilobular hypertrophy in 15 / 50 males and 7 / 50 females at 163 / 302 mg/kg bw/d) were concluded by the dossier submitter to be treatment related. There were no other dose-related changes in non-neoplastic histopathology findings.

In terms of neoplastic findings at 24 months, there were slight increases in the incidence of malignant lymphoma (haemolymphoreticular system) in males and of adenocarcinoma in the uterus of females in the high-dose groups compared with the controls. Neither of these was statistically significantly changed from the controls in any treatment group. The total numbers of primary neoplasms (73 in controls versus 65), benign neoplasms (62 in controls versus 53), and malignant neoplasms (11 in controls versus 12) were comparable between control and high-dose females. In males, the total numbers of primary (67 versus 42), benign (53 versus 35) and malignant neoplasms (14 versus 7) was 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 incidences of tumours in the haemolymphoreticular system and the uterus, together with historical control data, are presented in the table below.

Table 22. Incidence of neoplastic findings in rats administered mefentrifluconazole for 2 years

	Males				Females			
Dose level [mg/kg bw/d]	**0**	**4**	**25**	**163**	**0**	**6**	**38**	**302**
No. of animals	50	50	50	50	50	50	50	50
HEMOLYMPHRET SYSTEMexam.	50	50	50	50	50	10	10	50
Lymphoma, malignant		2 (4%)	2 (4%)	3 (6%)			1 (2%)	
Sarcoma, histiocytic	1	1	2				1	
UTERUS exam.	—				50	38	37	50
Adenocarcinoma, endometrial					1 (2%)	7 (18%)	3 (8%)	5 (10%)
Schwannoma, malignant						1	1	1
Adenoma, endometrial					1	1		2

Statistical analysis: *: $p \leq 0.05$, **: $p \leq 0.01$ (Fisher's Exact test, 1-sided)
Historical control data (24-month data from 12 studies in Wistar rats, started: Jan-2003 to Feb-2013)
(Survival rate: males 74 – 100%, females 58 – 100%)
♂ Malignant lymphoma: Mean: 2.5% (15/600); min.: 0% (0/50); max. 6% (3/50)
♀ Uterus, Adenocarcinoma, endometrial Mean: 16.2% (97/600); min.: 2% (1/50); max: 30% (15/50)

The applicant under Regulation 1107/2009 provided historical control data for these tumour types from the same test facility and conducted in the same strain of rat spanning a period of ten years before the conduct of the mefentrifluconazole study. The dossier submitter has narrowed the time-frame of these data to studies that were conducted within five years of the mefentrifluconazole study (dosing period during 2013 to 2015) (see below).

Malignant lymphoma of haemolymphoreticular system

The incidence of malignant lymphoma in the high-dose male group (6 %) was at the uppermost boundary of the provided historical control range but slightly above that of the most relevant range, correlating to one additional animal in the group of 50 presenting with this tumour. A clear dose-response relationship in the mefentrifluconazole study was not evident. Survival to termination of the study in the high-dose group males

was high (47/50) and was comparable between the low- and mid-dose groups, so the lack of a clear dose-response relationship is not explained by early deaths of animals in the high-dose group or differences between groups.

Table 23. Summary of relevant historical control data – tumours of the haemolympho-recticular system in male Wistar rats

Study duration	Application	Animal numbers	Lymphoma		Histiocytic sarcoma		Lymphoma		Survival rate
			No.	%	No.	%	Surv	Dec	
2009-2011	Drinking	50	2	4	0	0	0	2	80 %
2006-2008	Feeding	50	2	4	0	0	1	1	76 %
2007-2009	Feeding	50	2	4	0	0	0	2	88 %
2009-2011	Feeding	50	2	4	0	0	0	2	76 %
2013-2015	Feeding	50	0	0	1	2	0	0	68 %
Sum		250	8						
Mean				3.2					

Surv = survivors. Dec = decedents

The three male animals in the high-dose group in which malignant lymphoma was diagnosed were all decedents, as were the two affected males in the low-dose group. In contrast, both of the affected animals in the mid-dose group survived to termination of the study (day 735, week 105). The animals in the low-dose group that were diagnosed with lymphoma died on days 539 (week 77) and 238 (week 34), whereas those in the high-dose group survived until days 705 (week 100), 678 (week 97) and 708 (week 101). No cases of malignant lymphoma were detected at the 12-month interim sacrifice. The historical control data shows that all but one animal with the same tumour type died before the scheduled sacrifice.

Uterine adenocarcinoma

The incidence of uterine adenocarcinoma in all the exposed groups (10 % in the high-dose group) was within the historical control range of the most relevant studies, even though survival to termination of the study in the high-dose group (90 %) exceeded survival rates of the historical controls. One of the high-dose females in which uterine adenocarcinoma was diagnosed was a decedent, which was also a common occurrence in the historical control data. Moreover, the incidence in the mefentrifluconazole study did not show a dose-response relationship.

Table 24. Summary of relevant historical control data – uterine tumours in female Wistar rats

Study duration	Application	Animal numbers	Adenoma		Total Adenocarcinoma		Adenocarcinoma		Survival rate
			No.	%	No.	%	Surv	Dec	
2009-2011	Drinking	50	0	0	9	18	8	1	88 %
2006-2008	Feeding	50	1	2	14	28	9	5	74 %
2007-2009	Feeding	50	0	0	8	16	6	2	74 %
2009-2011	Feeding	50	0	0	10	20	7	3	72 %
2013-2015	Feeding	50	0	0	6	12	4	2	84 %
Sum		250			47				
Mean						18.8			

Surv = survivors. Dec = decedents

Carcinogenicity study in mice

In an 18-month carcinogenicity study in C57BL/6JRj mice, mefentrifluconazole was administered in the diet at dose levels of 0, 20 and 50 ppm (both sexes), 200 ppm (males) and 250 ppm (females). These concentrations corresponded to mean intakes of 3.5, 9.1 and 36 mg/kg bw/d in males and 4.9, 12.6 and 61.5 mg/kg bw/d in females.

There were no overt clinical signs of toxicity in any test group. Survival to the end of the study was not affected by exposure to mefentrifluconazole and exceeded 80 % in all groups. Necropsy of decedents did not show any evidence of a treatment-related cause of death. Treatment-related reductions in body weight and body-weight gain occurred in males at 200 ppm / 36 mg/kg bw/d from week 11 onwards and in females at 250 ppm / 61.5 mg/kg bw/d from week 7 onwards (the final body weight of females at this dose was reduced by 12 %); at the mid-dose of 50 ppm / 12.6 mg/kg bw/d, body weights and body-weight gains of females were reduced from week 34. Food consumption was unaffected in males at all doses, whilst in females at the high dose, food consumption was decreased in weeks 6 – 26, followed by normal food intake for the remainder of the study. At doses of 12.6 mg/kg bw/d and below, food consumption was not affected in females. There were no treatment-related changes in haematology parameters.

Gross necropsy did not reveal any treatment-related changes. Relative liver weights were increased at all doses in males (by 12 %, 18 %, 42 % at 3.5, 9.1, 36 mg/kg bw/d) and at the high-dose level in females (by 57 %). In the high-dose-group females and the mid- and high-dose males, the increases in liver weight were adverse, since increased incidences and severity of fatty change were noted at the same doses. A dose-related decrease in absolute kidney weights was reported only in males; when this was adjusted for body weight, only the change at 200 ppm / 36 mg/kg bw/d was statistically significant. However, histopathology changes (decreased incidence of tubular vacuolation) were observed in the kidneys of males of the mid- and high-dose groups; hence the dossier submitter considers the reduced absolute kidney weight at 50 ppm / 9.1 mg/kg bw/d to be a real and not artefactual effect. Adrenal-gland weights were increased in the high-dose males (absolute and relative) and females (relative). In males, there were no associated macroscopic or microscopic findings. However, in females, there were histopathology changes (increased incidence of eosinophilic cytoplasmic change and increase in size of individual eosinophilic cells) that, together with the weight increase, were consistent with mild repetitive stress of the animals.

Non-neoplastic microscopic changes were noted in the liver and thyroid glands (males and females), kidneys (males) and in the adrenal glands (females).

In the liver, a diffuse fatty change of hepatocytes was observed in most animals and was characterised by a microvesicular cytoplasmic change. Although there was no difference in total incidence of this change compared with the control animals, the severity was slightly increased in males treated at 9.1 and 36 mg/kg bw/d and in females at 61.5 mg/kg bw/d. In the same dose groups, the incidence and severity of macrovesicular fatty change was increased; this finding was characterised by the presence of large vesicles within the hepatocytes. The livers of two animals per group per sex were stained with Oil-Red-O to confirm that the vacuoles in the liver represented fat (fatty change); all were stained, indicating a positive result. In addition to the fatty changes, eosinophilic cytoplasmic inclusions in hepatocytes (centrilobular distribution) were recorded in the majority of the males at 36 mg/kg bw/d and hepatocellular single cell necrosis (minimal severity) occurred in 20 % of the high-dose females.

Table 25. Incidence of non-neoplastic liver findings in mouse carcinogenicity study

	Males				Females			
Dose level [mg/kg bw/d]	**0**	**3.5**	**9.1**	**36**	**0**	**4.9**	**12.6**	**61.5**
No. of animals	50	50	50	50	50	50	50	50
LIVER examined	50	50	50	50	50	50	50	50
Fatty change, diffuse (microvesic.)	48	46	47	48	40	44	38	43
Minimal (grade 1)	7	5	2	1	10	11	5	3
Slight (grade 2)	30	15	3	3	29	32	21	6
Moderate (grade 3)	10	24	33	35	1	1	11	8
Marked (grade 4)	1	2	9	9			1	26
Mean severity grade	*<2.0>*	*<2.3>*	*<2.9>*	*<3.0>*	*<1.4>*	*<1.6>*	*<1.7>*	*<2.9>*
Fatty change, macrovesicular	23	16	**35***	**46****	21	16	22	**39****
Minimal (grade 1)	20	14	8	13	14	13	7	3
Slight (grade 2)	3	2	14	16	7	3	12	2
Moderate (grade 3)			11	13			3	11
Marked (grade 4)			2	4				23
Mean severity grade	*<0.5>*	*<0.4>*	*<1.5>*	*<2.0>*	*<0.6>*	*<0.4>*	*<0.8>*	*<2.6>*
Eosinophilic inclusions, centrilob.				**38****				
Single cell necrosis, increased							1	**10***
Minimal							1	10

Statistical analysis: Fisher's Exact Test (1-sided); * : p≤0.05; ** : p≤0.01

In the thyroid, the incidence of follicular-cell hyperplasia of males at 36 mg/kg bw/d (statistically significant) and of females at 61.5 mg/kg bw/d (not statistically significantly different from control females) was increased. One of the criteria for the diagnosis of follicular cell hyperplasia was piling up of the epithelium into the lumen with the presence of (a) stromal component(s). The follicular cell hyperplasia incidences observed in the male and female high-dose groups were above the date-relevant historical control range of the test facility, although it is noted that this was also the case in the female control group.

Table 26. Non-neoplastic thyroid-gland findings in mouse carcinogenicity study

	Males				Females			
Dose level [mg/kg bw/d]	**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 ≤ 0.05, **: p ≤ 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)

In the kidney, a dose-dependent decrease in the incidence of tubular vacuolation in males was reported, which correlated with the decreased kidney and body weights at 9.1 and 36 mg/kg bw/d. This finding was considered by the study authors to be a subtle change that was not indicative of a degenerative process, but more likely an expression of increased (energy-consuming) excretion activity rather than an adverse effect.

Table 27. Incidence of non-neoplastic kidney findings in mouse carcinogenicity study

	Males				Females			
Dose level [mg/kg bw/d]	**0**	**3.5**	**9.1**	**36**	**0**	**4.9**	**12.6**	**61.5**
No. of animals	50	50	50	50	50	50	50	50
KIDNEY examined	50	50	50	50	50	5	10	50
Tubular vacuolation	42	42	**34***	**6****	0	0	0	0

Statistical analysis: Fisher's Exact Test (1-sided); * : p≤0.05; ** : p≤0.01

In the adrenal glands, a statistically significant increase in the incidence of eosinophilic cytoplasmic change was observed in the high-dose females. This finding was characterised by diffuse eosinophilic appearance of the cortical-cell cytoplasm of all three zones, together with a minimal to slight diffuse size increase of the individual cells. No signs of degenerative processes were observed in conjunction with this finding. The incidence of cortical hypertrophy without cytoplasmic changes in exposed animals was low and comparable with the control females.

Table 28. Incidence of selected non-neoplastic adrenal-gland findings

	Males				Females			
Dose level [ppm]	**0**	**3.5**	**9.1**	**36**	**0**	**4.9**	**12.6**	**61.5**
No. of animals	50	50	50	50	50	50	50	50
ADRENAL GLAND examined	50			50	50	50	50	50
Cytoplasmic change, eosinophilic	0			0	2	6	3	**20****
Hypertrophy, cortex, diffuse	1			0	6	6	**0***	4
Minimal					3	3		2
Slight	1				3	3		2

Statistical analysis: Fisher's Exact Test (1-sided); * : p≤0.05; ** : p≤0.01

In terms of neoplastic findings, there were very low incidences of follicular-cell adenomas of the thyroid gland and hepatocellular adenomas and carcinomas of the liver (see table below).

Table 29. Incidence of selected neoplastic findings in mouse carcinogenicity study

	Males				Females			
Dose level [mg/kg bw/d]	**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 exam.	50	50	50	50	50	50	50	50
Adenoma, follicular cell				2 (4 %)	1 (2 %)		1 (2 %)	3 (6 %)
LIVER exam.	50	50	50	50	50	50	50	50
Adenoma, hepatocellular	1 (2 %)	2 (4 %)	3 (6 %)					
Carcinoma, hepatocellular	1	1	1		1			

Historical control data (Test facility, four 18-month studies in C57BL/6JRj mice, started: 2013 - 2014)
Males: thyroid, follicular cell adenoma: Mean: 1.5% (3/198); min.: 0% (0/50); max. 2% (1/50)
Females: thyroid, follicular cell adenoma Mean: 2.6% (5/196); min.: 0% (0/50); max: 6% (3/47)

Historical control data (BASF, five 18-month studies in C57BL/6JRj mice, started: 1998 - 2007)
Males: thyroid, follicular cell adenoma: Mean: 1.2% (3/250); min.: 0% (0/50); max. 6% (3/50)
Females: thyroid, follicular cell adenoma Mean: 3.6% (9/250); min.: 0% (0/50); max: 8% (4/50)

Liver tumours

The adenomas and carcinomas in the liver of male mice of the low- and mid-dose groups were not treatment-related, since there were no such lesions in the high-dose group. All animals in the male high-dose group survived to termination of the study (one unscheduled death in the control males), and so the absence of tumours in the high-dose males was not due to the early deaths of those animals. There were no hepatocellular adenomas in female mice; the sole incidence of hepatocellular carcinoma was in a control female. There was therefore no evidence that mefentrifluconazole induced liver tumours in mice.

Thyroid-gland adenomas

The incidence of adenomas in the thyroid glands was slightly increased in the 200/250 ppm treated animals compared with the concurrent controls. The incidence was not statistically significantly changed compared with the control groups and was comparable to the background incidences in this strain of mice. The applicant provided two sets of historical control data. When compared with the control data of BASF, the incidence in males and females exposed to mefentrifluconazole was within the upper range; when compared with date-relevant data from the test facility that conducted the mefentrifluconazole study (dosing period 2013 – 2015), the incidence in males exposed to 36 mg/kg bw/d exceeded the upper range by one animal. The incidence in females was within the historical control range. The relevant historical control data are presented below.

Table 30. Summary of relevant historical control data – thyroid-gland findings in C57BL mice (18 months; same test facility as the mefentrifluconazole study)

In-life period	**HCD study 1 2013-2015**		**HCD study 2 2014-2015**		**HCD study 3 2014-2015**		**HCD study 4 2014-2015**		**Mefentri-fluconazole 36/61.5 mg/kg bw/d**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Survival rate (%)	*96*	*88*	*92*	*86*	*86*	*86*	*82*	*86*	*100*	*89*
THYROID GLANDS[a], follicular cell	50	50	49	47	49	50	50	49	50	50
Hyperplasia	9 18 %	3 6 %	22 45 %	13 28 %	16 33 %	12 24 %	14 28 %	6 12 %	37 74 %	26 52 %
Adenoma	1 2 %	0	1 2 %	3 6.4 %	0	0	1 2 %	2 4.1 %	2 4 %	3 6 %
Adenocarcinoma	0	0	0	2 4.3 %	0	0	0	0	0	0
Number of tumour-bearing animals	1	0	1	5	0	0	1	2	2	3

[a] = Number of tissues examined from each group

In females, the incidence of thyroid follicular-cell hyperplasia was not dose-related and was within the relevant historical control range. Follicular-cell hyperplasia was also not dose-related in females, since the incidence in mid-dose females was statistically significantly less than in the controls. No adenocarcinomas were detected in either sex. The two cases of thyroid follicular cell adenomas in high-dose males occurred in animals that survived to termination of the study (there were no unscheduled deaths in this group). In females, 1, 0, 1, 3 adenomas were present in animals at 0, 4.9, 12.6 and 61.6 mg/kg bw/d that survived to termination; therefore adenomas in the high-dose group did not result in early deaths. In all affected high-dose animals, adenomas occurred singly. Furthermore, in one of two males and two of three females, they were reported in the absence of thyroid follicular-cell hyperplasia. There was therefore no evidence that the adenomas occurred as a continuum of treatment-related pathological consequences, with a progression from hyperplasia. Moreover, the total number of neoplasms in all groups was comparable, with equal numbers in the control and high-dose males (13 in each group, in 12 animals each) and fewer in the high-dose females (20 in 18 animals) than in the

controls (27 in 21 animals). No animal in any treatment group had metastasis, whereas one was reported in a control female.

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

Two life-time studies are available, one in rats and one in mice, both of which are adequate to assess the carcinogenic potential in rodents of mefentrifluconazole. At the highest doses administered (163 / 302 mg/kg bw/d in rats, 36 / 61.5 mg/kg bw/d in mice, males and females, respectively), systemic toxicity was induced (> 10 % reduction in body weight and body-weight gain, liver toxicity) but long-term survival of the animals was not affected.

In rats, increases in tumour incidences compared with the concurrent controls were noted in the haemolymphoreticular system in males (malignant lymphoma) and the uterus of females (adenocarcinoma), although neither of these was statistically significant. A clear dose-response relationship was not evident in the malignant-lymphoma incidences, and the high-dose group incidence (6 %) was within the wider historical control range, exceeding the more recent historical control range (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 historical control data. 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 incidence of uterine adenocarcinoma 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 historical control range (12 – 28 %, mean 18.8 %) for this common tumour of aged female rats. The dossier submitter thus concludes that the increased incidence above the concurrent controls in the mefentrifluconazole study was incidental.

In mice, there was a very slight increase (not statistically significant) in the incidence of thyroid follicular-cell adenomas which, nevertheless, was within the historical control range for females and above the historical control range for males by only one animal. 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 dossier submitter considers 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 dossier submitter concludes that the thyroid tumours arose spontaneously. Mefentrifluconazole did not induce liver tumours in mice.

In both studies, the total number of primary, benign and malignant neoplasms was comparable between control and exposed groups.

Overall, therefore, mefentrifluconazole was not carcinogenic in rats or mice under the conditions of these studies.

10.9.2 Comparison with the CLP criteria

In long-term toxicity and carcinogenicity studies with mefentrifluconazole (a two-year combined chronic toxicity / carcinogenicity study in Wistar rats and an 18-month dietary carcinogenicity study in C57BL/6JRj mice), there was no evidence of treatment-related carcinogenicity in either species. Neither ‘sufficient’ nor ‘limited’ evidence of carcinogenicity in animals was demonstrated. There is no information on the carcinogenicity of mefentrifluconazole in humans.

10.9.3 Conclusion on classification and labelling for carcinogenicity

Not classified (conclusive but not sufficient for classification).

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

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:

	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
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 ≤ 0.05, **: p ≤ 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 their 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 concludes that mefentrifluconazole did not induce liver tumours. There is insufficient evidence to support a classification in category 2 based on 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**.

10.10 Reproductive toxicity

The reproductive toxicity of mefentrifluconazole has been investigated in a two-generation study in rats and developmental toxicity studies in rats and rabbits.

10.10.1 Adverse effects on sexual function and fertility

A two-generation study in rats is available to investigate the effects of mefentrifluconazole on sexual function and fertility.

Table 31. Summary table of animal studies on adverse effects on sexual function and fertility

Study **Species (strain),**	**Dose levels**	**Critical effects**
Rat (Wistar, Crl:WI(Han) Dietary administration with dose adjustment 25 / sex / group OECD 416 GLP Purity 98.8% [redacted] 2015c	0, 25, 75, 200 mg/kg bw/d	**Parental toxicity**
		F_0 generation ≥ 75 mg/kg bw/d: ↑ ALP (males & females), cholesterol (males), liver wt (males & females) 200 mg/kg bw/d: ↓ food consumption, body weight and body-weight gain (males & females) ↑ liver cell hypertrophy (males) 1 dam with total litter loss by PND 2; one pup of this litter showed signs of insufficient nursing (no milk in stomach); no findings in 5 other pups; remainder cannibalised.
		F_1 generation 200 mg/kg bw/d: ↓ food consumption, body weight and body-weight gain (males & females) ↑ ALP (males & females), urea & inorganic phosphate (males), triglycerides (females) ↑ liver weight (males & females), liver cell hypertrophy (15/25 males, minimal) 1 dam with total litter loss by PND 3; pups showed signs of insufficient nursing (reduced nutritional condition, no milk in stomachs) 1 dam with only stillborn pups; this dam showed poor general state and piloerection
		Fertility
		F_0- & F_1 generation No treatment-related adverse effect
		Offspring toxicity
		F_1 pups (200 mg/kg bw/d) ↓ live pups (PND 4) owing to 1 total litter loss ↓ pup weight and weight gain during lactation (& secondary organ-weight effects)
		F_2 pups (200 mg/kg bw/d) ↓ live pups (PND 0 and PND 4) owing to 1 dam with only stillborn pups and 1 dam with total litter loss ↓ pup weight and body-weight gain during lactation (& secondary organ-weight effects)

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

In a guideline-compliant two-generation study in rats, mefentrifluconazole was administered to groups of 25 / sex / dose in the food; the dietary concentrations were adjusted to obtain target dose levels of 0, 25, 75 and 200 mg/kg bw/d. At least 75 days after the beginning of treatment, F_0 animals were mated to produce an F_1 generation. Mating pairs (one male to one female) were from the same dose group and F_1 animals selected for breeding were continued in the same dose group as their parents. The same group sizes and doses were repeated to produce the F_2 generation. Test diets that contained mefentrifluconazole were offered continuously throughout the study.

Parental toxicity

There were no treatment-related deaths in any group. One female of the 75 mg/kg bw/d group was sacrificed during the pre-mating period for reasons unrelated to mefentrifluconazole administration. There were also no treatment-related overt clinical signs in any F_0 or F_1 parental animal during the pre-mating or mating phases. Dam health during gestation and lactation is reported below.

Parental body-weight gain was impaired at 200 mg/kg bw/d in parental F_0 and F_1 animals. In F_0 males at this dose, body weights were statistically significantly below the concurrent control values from pre-mating day 13 onwards and remained so until the end of the study (up to 11 % reduction). The body-weight gain of these males was statistically significantly below the control values during major parts of the pre-mating period (up to 33 %) and consistently lower than the control during other study periods (mating, post-mating), albeit without statistical significance. In the high-dose F_0 females, body weights were consistently below concurrent controls throughout the study, and gained statistical significance during pre-mating days 48–55 (up to 5 % reduction), during the entire gestation period (up to 9 %) and during lactation days 1–14 (up to 13 % decrease). The body-weight gain of these females was statistically significantly below the control values during major parts of the gestation period (up to 24 %), although they generally gained more weight during lactation (23.7g, 26.6g, 34.5g. 44.9g at 0, 25, 75, 200 mg/kg bw/d, gain over lactation day 1 to 21). The F_0 females showed no clinical signs during gestation and lactation, apart from one high-dose dam (number 200) that had complete litter loss on post-natal day 2.

The body weights of the F_1 males at 200 mg/kg bw/d were statistically significantly below the control values from the beginning of pre-mating onwards and remained so until the end of the study (up to 12 % decrease). The body-weight change of these males was statistically significantly below the control values during major parts of the pre-mating period (up to 32 % lower) and during post-mating days 0-7 (a reduction of about 58 %). Also at 200 mg/kg bw/d, the body weights of the F_1 females were statistically significantly below the concurrent control values during the entire pre-mating, gestation and lactation periods (decreases of up to 11 %, 16 % and 17 %, respectively). The body-weight change of these females was below the control values during most of the pre-mating period (days 0–69, reduced by about 11%) and throughout gestation (up to 32 % lower). In contrast, these females generally gained more weight during lactation (28.2, 26.4, 29.9 and 36.5 g at 0, 25, 75 and 200 mg/kg bw/d, gain over lactation day 1 to 21). One high-dose F_1 female (number 379) showed severe poor general condition, piloerection and an inability to deliver on gestation day 23, followed by delivery of a still-born litter on gestation day 24; otherwise, there were no clinical signs during gestation and lactation.

The body weights and body-weight gains of the F_0 and F_1 parental animals at 25 and 75 mg/kg bw/d were comparable to the concurrent-control groups throughout the study.

Food consumption of the F_0 males at 200 mg/kg bw/d was statistically significantly below the control values during the entire pre-mating period (up to 11 %), during mating days 9-14 (about 10 %) and throughout the post-mating period (up to 10 %). In F_0 females at 200 mg/kg bw/d, it was also consistently below the control values throughout the study, although the difference gained statistical significance only during pre-mating days 63-69 (about 6 %), during the entire gestation period (up to 15 %) and during lactation days 7-14 (about 12 %). The high-dose F_1 males had consistently and statistically significantly reduced food consumption during the entire pre-mating (up to 9 %) and post-mating (up to 10%) periods. Food consumption in both sexes and generations was unaffected at 25 and 75 mg/kg bw/d.

Oestrous cycle determinations were evaluated by daily analysis of vaginal smears for all F_0 and F_1 female parental rats for a minimum of 3 weeks prior to mating and were continued throughout the mating period until the female exhibited evidence of copulation. Additionally, the stage of the oestrous cycle for each female was determined at scheduled sacrifice. The investigations revealed the occurrence of regular cycles in all groups of both generations. In the F_0 females, the mean oestrus-cycle duration was 4.1, 4.2, 4.1 and 4.2 days at 0, 25, 75 and 200 mg/kg bw/d, respectively. In the F_1 females, the mean duration was 4.1, 4.0, 4.1 and 4.6 days at 0, 25, 75 and 200 mg/kg bw/d, respectively. The value for the F_1 high-dose females was statistically significantly ($p < 0.05$) above the control value, by half a day, and the number of cycles within the observation period was accordingly lower (3.48 versus 4.32 in controls, $p < 0.01$). The duration of cycles was within the historical control range of the test facility [6 studies (2010-2014): 3.99 – 4.8 days, mean 4.33]. Furthermore, neither time to pairing nor pairing success were affected. Therefore, the dossier submitter does not consider this apparent slight lengthening of the oestrus cycle in the high-dose group to be evidence of an adverse effect.

Clinical chemistry findings in adults comprised increased ALP in both sexes in each generation, and increased cholesterol in F_0 males, urea in F_1 males and triglyceride in females. A decrease in total bilirubin in males and females, in the absence of signs of anaemia, was likely to be a reflection of increased phase-II hepatic metabolism with a consequent increased biliary excretion of bilirubin, and hence was an adaptive change.

The majority of noted organ weight changes in adults were a reflection of the decreased terminal body weights. For example, the absolute adrenal, spleen (males), testes, cauda epididymis, ovaries, kidney (females) and pituitary gland (females) were decreased, but when adjusted for body weight showed no difference from controls. In both sexes, the relative brain weight was increased in the same magnitude as the decrease in body weight; this and the other mentioned weight changes were thus not regarded by the dossier submitter to be organ-specific effects. Treatment-related increases in absolute and relative liver weights were recorded in males and females at 75 and 200 mg/kg bw/d in both generations. Neither absolute nor relative weights of the prostate (males), thyroid (either sex), spleen (females) and uterus were statistically significantly changed in either generation.

There were no treatment-related gross pathology findings in the F_0 and F_1 parental animals. Histopathology revealed a treatment-related, minimal centrilobular hypertrophy in 15 / 25 F_0 males and 15 / 25 F_1 males at 200 mg/kg bw/d. There were no other treatment-related microscopic findings. A differential ovarian follicle count on the F_1 females showed that there were no biologically or statistically significant differences in the numbers of primordial, growing and combined incidence of follicles between the controls and the high-dose group.

Male reproductive parameters

The male mating index[2] was 100 % in all test groups in both generations. The male fertility index[3] for the F_0 parents was 100 % in all test groups. For the F_1 parental males, two high-dose males (numbers 283 and 293) did not generate F1 pups, so that the male fertility index ranged between 92 % and 100 %. Extensive historical control data from the test facility (33 studies), dating from 2008 to 2015, was provided to argue that this finding reflected the normal range of biological variation inherent in the strain of rats used (range 80-100, mean = 91.8); the index in the high-dose group was thus identical to the mean of the historical-control data. The dossier submitter notes that the mefentrifluconazole study was conducted in 2013, and so the date ranges of the historical control data are appropriate. The two males in question did not show gross necropsy,

[2] $\text{Male mating index [\%]} = \frac{\text{number of males with confirmed mating *}}{\text{number of males placed with females}} \times 100$

* defined as females with vaginal sperm or with implants in utero

[3] $\text{Male fertility index [\%]} = \frac{\text{number of males proving their fertility *}}{\text{number of males placed with females}} \times 100$

* defined as females with implants *in utero*

histopathological findings or changes in sperm quality that would suggest infertility. The dossier submitter thus considers the finding in 2 / 25 males, only in the F_1 generation and within the historical-control range, to be incidental.

Table 32: Reproduction parameters of male rats treated with mefentrifluconazole

Parental generation	**F_0**				**F_1**			
Dose level [mg/kg bw/d]	**0**	**25**	**75**	**200**	**0**	**25**	**75**	**200**
- # animals per group	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**
- # males placed with females	25	25	24	25	25	25	25	25
- # males mated	25	25	24	25	25	25	25	25
- Male mating index [%]	100	100	100	100	100	100	100	100
- # mated females pregnant	25	25	24	25	25	25	25	23
- Male fertility index [%]	100	100	100	100	100	100	100	92

Statistical analysis: Fisher's Exact test (1-sided -); *: p< 0.05; **: p<0.01
Historical control data [33 studies run 2008-2015 at test facility with Wistar rats (supplier: Charles River)]
- Male fertility index [%]: 80-100, mean = 91.8

Sperm analyses, which investigated sperm motility and determined the incidence of sperm head counts in the testis as well as the percentage of abnormal sperm in the testis and cauda epididymis, did not indicate any effects of treatment in F_0 or F_1 males.

Female reproductive parameters

F_0-generation parental females

Mefentrifluconazole did not adversely affect reproduction or delivery of the F_0-generation parental females. The female mating index[4] was 100 % in all groups. All female F_0 rats delivered pups or had implants *in utero*. The fertility index[5] was thus 100 % in all test groups. The mean duration of gestation was slightly increased in the high-dose group (22.4 compared with 22.1 in controls [$p \leq 0.05$]), although this represented a difference from the control group of less than half a day; furthermore, all values were within the historical control range of the test facility (33 studies, 2008 to 2015: 21.9 to 22.9). Parturition appeared to proceed normally in all animals of the high-dose group; clinical signs were not reported in the dams, and the live-birth index[6] of the high-dose group was comparable with the controls, as was the number of still-born pups. Generally, the post-natal pup survival of the high-dose group was also comparable with the controls (see section on offspring toxicity), apart from one dam (number 200), which delivered eleven live pups on gestation day 23 (none dead) but had total litter loss on post-natal day 2. This dam had markedly lower food consumption on post-natal days

[4] $\text{Female mating index [\%]} = \frac{\text{number of females mated *}}{\text{number of females placed with males}} \times 100$

* defined as the number of females with vaginal sperm or with implants in utero

[5] $\text{Female fertility index [\%]} = \frac{\text{number of females pregnant *}}{\text{number of females mated * *}} \times 100$

* defined as the number of females with implants in utero

** defined as the number of females with vaginal sperm or with implants in utero

[6] $\text{Live birth index [\%]} = \frac{\text{number of liveborn pups at birth}}{\text{total number of pups born}} \times 100$

1 – 4 (16.5 g) than the group means (32.0, 30.8, 32.6 and 28.8 g at 0, 25, 75 and 200 mg/kg bw/d, respectively). The gestation index[7] was 100 % in all groups.

The mean number of implantation sites in the F_0 parental females was comparable between all groups. Furthermore, there were no statistically significant differences in post-implantation loss between the groups nor the litter size in the mid- and high-dose groups (the dossier submitter considers a slightly lower value only in the low-dose group to be incidental).

F_1-generation parental females

The female mating index of the F_1 parental animals was 100 % in all groups. All female F_1 rats delivered pups or had implants *in utero* except for high-dose female number 383 (mated with male number 293) and high-dose female number 393 (mated with male number 283); these females were sperm-positive but did not become pregnant. There were no corroborative gross or histopathological findings in the sexual organs of these two females and the time-to-mating was also unremarkable. The fertility index in the high-dose group was thus 92.0 %, compared with 100 % in the other groups. This value for the high-dose group was within the range of the date-relevant and extensive historical control data of the test facility in the same strain (33 studies, 2008 to 2015: range 80 to 100 %). The mean duration of gestation was similar in all test groups (i.e., between 22.0 and 22.2 days). The gestation index of the high-dose group (91.3 %) was slightly below that of the other groups (100 %) owing to two females (number 379: 6 still-born pups; number 383: 1 implantation site, no pups present) that were pregnant but did not deliver any live pups. This value was within the historical control range of the test facility over an appropriate time-frame and in the same strain (33 studies 2008-2015: range 87.5 to 100 %). Within this historical-control data, four females had entirely still-born litters. Therefore, it is not clear that mefentrifluconazole was responsible for the still-born litter in the present study.

The mean number of implantation sites per dam was slightly but statistically significantly lower in the high-dose-group F_1 parents (mean 10.0, range 1 to 14, compared with mean 12.0, range 9 to 16 in the controls). The mean of the high-dose group was affected by one female (number 388) that contained only one implant. Notwithstanding, the mean value of the high-dose group was within the historical-control range of the test facility (33 studies, 2008-2013: range 9.4 to 14.0). A dose-related change was also not seen, since the mean number of implantation sites in the mid-dose group was higher than that in the control group. Overall, therefore, the dossier submitter considers that the slightly lower value in the high-dose group reflected biological variation and was not a treatment-related effect. Although there were slight increases in post-implantation loss in the mid- (7.1 %) and high-dose (8.9 %) groups compared with the controls (2.4 %), these were also most likely to be a reflection of normal biological variation and were well within the historical control range (33 studies, 2008-2013: range 0.9 to 17.7). Also, the value of the high-dose group (which was not statistically significantly different from the control value) was strongly influenced by the female (number 388) with only one implant; this implant was resorbed, which resulted in a post-implantation loss of 100 % for this animal. Exclusion of this animal from the calculation would lead to a group mean of 4.8 %.

As a consequence of the fewer implants, the mean number of F_2 pups delivered per dam (excluding those that were not pregnant or did not deliver) was statistically significantly decreased in the high-dose group (9.9 compared with 11.9 in the controls) but without a clear dose-response relationship. Furthermore, since the mean value was within the historical control range of the test facility (9.2 – 13.4), there was no evidence that mefentrifluconazole exposure affected the number of pups born per dam. The live-birth index was also unaffected and within the historical control range of the test facility at all doses, with six of the nine still-born pups at 200 mg/kg bw/d being from one litter, the dam of which was obviously unwell.

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[7] $\text{Female gestation index [\%]} = \frac{\text{number of females with live pups on the day of birth}}{\text{number of females pregnant *}} \times 100$

* defined as the number of females with implants in utero

Table 33: Reproduction parameters of female rats exposed to mefentrifluconazole

Parental generation	**F_0**				**F_1**			
Dose level [mg/kg bw/d]	**0**	**25**	**75**	**200**	**0**	**25**	**75**	**200**
- # animals per group	25	25	25	25	25	25	25	25
- # females placed with males	25	25	24	25	25	25	25	25
- # females mated	25	25	24	25	25	25	25	25
- Female mating index [%]	100	100	100	100	100	100	100	100
- # females pregnant	25	25	24	25	25	25	25	23
- Female fertility index [%]	100	100	100	100	100	100	100	92
Pre-coital interval [mean days]	2.8	2.4	3.0	2.8	3.0	3.0	2.5	2.8
Duration of gestation [mean days]	22.1	22.2	22.2	**22.4***	22.2	22.0	22.0	22.2
Implantation sites, total	307	284	288	295	300	285	308	229
- per dam	12.3	11.4	12.0	11.8	12.0	11.4	12.3	**10.0***
Post-implantation loss [mean %]	3.9	5.5	1.3	5.2	2.4	5.0	**7.1****	8.9
Females with live-born	25	25	24	25	25	25	25	21
- with still-born pups	1	1	0	1	1	3	2	3
- with all still-born	0	0	0	0	0	0	0	1
- Gestation index [%]	100	100	100	100	100	100	100	91.3
Pups delivered	297	267	284	277	298	269	285	217
- per dam	11.9	**10.7***	11.8	11.1	11.9	10.8	11.4	**9.9****
- live-born	296	266	284	274	295	263	283	208
- still-born	1	1	0	3	3	6	2	9
- Live-birth index [%]	99.7	99.6	100	98.9	99.0	97.8	99.3	95.9

Statistical analysis: *: p< 0.05; **: p<0.01

Historical control data [33 studies run 2008-2015 at test facility with Wistar rats (supplier: Charles River)]

- Duration of gestation: 21.8 – 22.9 days
- Gestation index: 87.5 – 100 %
- Implantation sites/dam: 9.4 – 14.0
- Post-implantation loss [mean %]: 0.9 – 17.7
- Pups delivered/dam: 9.2 – 13.4
- Live-birth index [mean %]: 92.1 – 100

Offspring toxicity

The viability index of F_1 pups during early lactation (post-natal day 0-4) was similar across the groups, without statistically significant differences. The slightly lower value in the high-dose group (93.5 %, compared with 99.1 % in the controls), resulting from more dead pups (18 vs. 3 in the controls), was a consequence of one litter (dam number 200) in which 11 pups died or were subject to cannibalism; the one pup that could be examined had indications of improper nursing (empty stomach). The remaining seven dead pups (two found dead, five cannibalised) at 200 mg/kg bw/d originated from the litters of six additional F_0 dams. The viability indices in the study were inside the range of historical controls (33 studies conducted 2008-2015: 89.4 % – 100 %). Since the slightly reduced viability index at 200 mg/kg bw/d was heavily influenced by one litter, at a dose at which maternal toxicity was evident (mean body weight reduced by up to 13 %) and within the historical control range, the dossier submitter does not consider that this constitutes evidence of a specific effect on post-natal survival. The lactation index indicated that pup survival between post-natal days 4 and 21 was high in all the groups.

The viability index of the F_2 pups indicated that pup survival during early lactation was similar in all the groups, with no statistically significant differences. The slightly lower value at 200 mg/kg bw/d was explained by a higher number of pups dying during post-natal days 1–4 (15 vs. 1 in the controls). Eleven of the 15 dead pups came from one litter (dam number 397), and were not properly nourished during post-natal day 1–3, as became evident by a severely reduced nutritional condition and the absence of milk in the stomach of the investigated

pups. Consequently, this animal lost its entire litter within the first three days after birth. The food consumption of this dam during post-natal days 1–4 (13.3 g) was notably lower than the group means (33.5, 30.2, 33.3 and 27.2 g at 0, 25, 75 and 200 mg/kg bw/d). The remaining four dead pups, which were subject to cannibalism, originated individually from the litters of four F_1 dams. The lactation index indicated that pup survival between post-natal day 4 and 21 was high in all the groups.

There was no evidence of a substance-related effect on the sex ratio in either generation.

Table 34: Pup survival and sex ratio

Parental generation	**F_0**				**F_1**			
Dose level [mg/kg bw/d]	**0**	**25**	**75**	**200**	**0**	**25**	**75**	**200**
Number of litters	25	25	24	25	25	25	25	22
- with live-born pups	25	25	24	25	25	25	25	21
- with still-born pups	1	1		1				1
Pups live-born	296	266	284	274	295	263	283	208
Pups found dead (day 1-4)	1	2		10			1	2
Pups cannibalized (day 1-4)	2			8	1	1	2	13
Pups PND 4 (pre-cull)	293	264	284	256	294	262	280	193
- Viability index [%]	99.1	99.4	100	93.5	99.7	99.6	99.0	93.6
Pups culled day 4	99	73	92	69	95	66	81	38
Pups PND 4 (post-cull)	194	191	192	187	199	196	199	155
Pups found dead (day 5-21)								
Pups cannibalized (day 5-21)			1			1		1
Pups PND 21	194	191	191	187	199	195	199	154
- Lactation index [%]	100	100	99.0	100	100	99.5	100	99.4
Sex ratio [% live males], PND 0	47.7	49.5	51.3	49.2	49.4	44.4	52.8	50.2
Sex ratio [% live males], PND 21	47.6	50.7	47.8	50.2	48.9	48.0	50.8	48.7

Statistical analysis, viability and lactation indices: Wilcoxon with Bonferoni-Holm (1-sided -), sex ratio: Wilcoxon test (2-sided); * $p \leq 0.05$, ** $p \leq 0.01$

No substance-related clinical observations were apparent in the F_1 pups. Of the F_2 pups, six from a dam dosed with 200 mg/kg bw/d mefentrifluconazole (number 397) showed evidence of a reduced nutritional condition and an absence of milk in the stomach on post-natal days 1 and 2. There were no clinical signs of toxicity in any of the other F_2 pups.

Male pups were investigated for the presence of nipples and areolae on post-natal days 12 and 20. At post-natal day 12, there was no dose-related effect on the apparent number and percentage of male F_1 or F_2 pups having areolae. By post-natal day 20, no areolae were detected in any of the male pups of either generation.

Anogenital distance was measured on post-natal day 1. There were no dose-related effects on anogenital distance or index[8] in the male or female F_1 pups. A slightly higher index in the high-dose group was likely to be an incidental finding, since a dose-related change in the index was not evident and the distance was very slightly less than that of the controls. In the F_2 male pups, the index was apparently higher than the controls in the high-dose group; this was a consequence of the lower body weight, since the actual distance was identical to that of the controls. The anogenital distance of the F_2 female pups was slightly below the control in all

[8] $\text{Anogenital index} = \frac{\text{anogenital distance [mm]}}{\text{cubic root of pup weight [g]}} \times 100$

mefentrifluconazole-exposed groups. However, as the index was unchanged and very close to the controls in all the exposed groups, these minimal differences were unlikely to be related to treatment.

At 200 mg/kg bw/d, lower F_1 pup body weights were noted on post-natal day 1 (about 9 % below controls). Pup body weights remained lower until weaning (about 10 % below controls on post-natal day 21). Accordingly, mean body-weight change was below the controls in the high-dose F_1 pups (by up to 15 %) throughout lactation. There were no treatment-related changes in F_1 pup body weights/body-weight change at 75 and 25 mg/kg bw/d.

In the F_2 pups at 200 mg/kg bw/d, lower pup body weights were noted on post-natal day 1 (about 7 % below controls). Pup body weights remained lower until weaning (about 14 % below controls on post-natal day 21). Overall, the mean body-weight change was below the concurrent controls in the high-dose F_2 pups (up to 19 % lower) throughout lactation.

Pup organ weights (brain, spleen and thymus) were measured from one male and one female in each litter on post-natal day 21 (weaning). The body weights at this time were used to calculate relative organ weights. The mean absolute brain (-2.9 %), spleen (-12.7 %) and thymus (-12.6 %) weights of male and female F_1 pups from the high-dose group were decreased. The relative brain weights of the high-dose male and female F_1 pups were increased by 9.1 %; there were no significant differences in the relative thymus or spleen weights between control and test groups. In the high-dose F_2 pups, absolute brain (-3.5 %), thymus (-16.2 %) and spleen (-17.6 %) weights were statistically significantly decreased. The relative brain weights of F_2 pups were increased by 13.1 % at 200 mg/kg bw/d; the relative spleen and thymus weights were not statistically significantly different from the controls at any dose. The dossier submitter considers the observed organ weight changes in both generations to be secondary to the lower pup body weights in the high-dose group.

Upon necropsy of the pups, one F_1 pup of litter 200, which was entirely lost, had an empty stomach; nothing abnormal was detected in five other pups of this litter. The remaining five pups of the litter were subject to cannibalism before they could be examined. In the F_2 pups, dilated renal pelves were found in 23 pups at 200 mg/kg bw/d (6 males, 17 females from 9 litters) compared with three affected female pups from three litters in the control group. The finding was treatment related but probably secondary to the general delay in development of the high-dose pups (up to 19 % decrease in body-weight gain); the study authors assumed this to be a largely reversible effect. All other findings in both generations occurred spontaneously across all the groups.

Table 35: Incidence of dilated renal pelvis in F_1 and F_2 pups

	Male pups				**Female pups**			
Dose [mg/kg bw/d]	**0**	**25**	**75**	**200**	**0**	**25**	**75**	**200**
F_1 pups								
Animals examined	119	107	121	109	127	107	110	117
Renal pelvis, dilated	3 (3)	2 (2)	5 (4)	2 (2)	1	3 (3)	3 (3)	3 (3)
F_2 pups								
Animals examined	145	120	148	112	152	149	132	105
Renal pelvis, dilated		1	1	6 (5)	3 (3)	2 (2)	1	17 (9)

( ) values in brackets give litter incidence

Sexual maturation was determined in each male and female F_1 pup that was selected to become an F_1 parent. The mean age at which females reached sexual maturity was slightly delayed in the high-dose pups (31.8 days compared with 30.0 days in controls), although there wasn't a clear dose-response relationship, with there being a statistically significant delay also in the low-dose (30.9 days) but not the mid-dose group (30.2 days). The days to opening were within the relevant historical control range in all the groups (15 studies, range 29.5 to 31.9); therefore, the dossier submitter does not regard this as evidence of a specific adverse effect. The age of preputial separation in male pups was not affected even in the high-dose group.

10.10.3 Comparison with the CLP criteria

The potential of mefentrifluconazole to adversely affect fertility, pregnancy outcome and post-natal offspring survival has been investigated in a guideline-compliant two-generation reproduction study in Wistar rats.

Parental toxicity was evident in the high-dose group (200 mg/kg bw/d) as consistent reductions in food consumption, body weights and changed clinical-chemistry parameters that, together with the increased liver weights, indicated some disruption of normal liver function. Some evidence of liver dysfunction was also evident in the mid-dose group (75 mg/kg bw/d).

In terms of fertility, the male and female mating index was 100 % in all groups in both generations, as was the fertility index in the F_0 parents. Two F_1 male/female pairs at 200 mg/kg bw/d failed to result in implants, but as there was no pathological explanation and the resultant fertility indices were within the historical control range, the dossier submitter concludes that this does not provide evidence of a treatment-related effect on fertility. In the F_1 generation there were some additional reductions in reproduction parameters in the high-dose group (implantation sites per dam, with a consequent reduction in pups delivered per dam) and a slight increase in post-implantation loss; again, these were within the historical control ranges and were likely to be a reflection of normal biological variation, possibly compounded to some extent by the maternal toxicity at this dose. Two high-dose females of the F_1 generation did not deliver any live pups; one of them (number 379) delivered six still-born pups, whilst the other (number 383) showed evidence of just one implantation site but no pups. These two females were responsible for the slight reduction in the gestation index at this dose that was, nevertheless, not statistically significant and was within the historical control range. Overall, there was no evidence of intra-uterine embryo- or fetal-lethality, since the post-implantation loss in the high-dose group in both generations was similar to the concurrent and historical controls.

The duration of gestation was very slightly but statistically significantly increased in the F_0 parent females; the increase represented only half a day. Within the F_0 generation, delivery appeared to proceed normally; there were no consequences on the number of live births or post-natal survival, apart from one dam that delivered on gestation day 23 and had total litter loss on post-natal day 2. However, there is no information to link the litter loss to the delay in parturition, and this occurrence only in one dam does not provide evidence of a treatment-related effect on pregnancy outcome. The mean duration of gestation was not affected in F1 dams. It is noted, however, that one of the F_1 dams (number 379) appeared to show normal condition on gestation day 22, but on gestation day 23 had severe general condition, piloerection and was unable to deliver, with the eventual delivery of only still-born pups on gestation day 24. Notwithstanding, this was an isolated occurrence and the dossier submitter thus concludes that mefentrifluconazole did not adversely affect gestation duration or parturition.

There was no evidence that mefentrifluconazole had a specific adverse effect on pup survival from post-natal days 1-4 or 4-21. Slight reductions in the viability index in both generations at 200 mg/kg bw/d, neither of which was statistically significant, were a consequence of single whole-litter losses; these appeared to be the result of inadequate nursing, arising from much reduced food intake of the respective dams. The overall lower food consumption and lower body weights early in lactation of the dams in the high-dose group affected development of the pups: in each generation, pup body weights of the 200 mg/kg bw/d group were reduced compared with the controls from post-natal day 1 and throughout lactation. An increased incidence of dilated renal pelves only in the high-dose-group F_2 generation was likely to be treatment related but not indicative of specific developmental toxicity; rather, it was a variation that was a secondary effect of delayed development, resulting from the lower body weights at this dose. This was also the most likely explanation for minor changes in some markers of development and sexual maturation.

Overall, the dossier submitter concludes 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) and offspring toxicity 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, a classification for adverse effects on sexual function and fertility is not proposed.

10.10.4 Adverse effects on development

The developmental toxicity of mefentrifluconazole has been investigated in rats and rabbits.

Table 36: Summary table of animal studies on adverse effects on development

Study Species	Dose levels	Critical effects
Rat oral prenatal developmental toxicity		
Rat (Wistar, Crl:WI(Han)) 25 females / group Vehicle: 1% carboxymethyl-cellulose OECD 414 GLP Purity 97.7% [redacted] 2015a	0, 50, 150, 400 mg/kg bw/d Gavage GD 6-19	**Maternal toxicity** At 400 mg/kg bw/d: ↓ food consumption (-8 %), body weight (-7 %) and body-weight gain (-34 % corrected weight) No adverse effects at other doses **Developmental toxicity** No treatment-related, specific adverse effects; all findings within historical control ranges
Rabbit oral prenatal developmental toxicity		
Rabbit (New Zealand White) 30-33 females / group Vehicle: 1% carboxymethyl-cellulose OECD 414 GLP Purity 95.5 % [redacted] 2015b (CA 5.6.2/2)	0, 5, 15, 25 mg/kg bw/d gavage GD 6-28	**Maternal toxicity** No treatment-related adverse effects (Dose selected on basis of range-finding studies, in which 2/3 non-pregnant rabbits at 50 mg/kg bw/d were sacrificed in a moribund condition.) **Developmental toxicity** No treatment-related, adverse effects

10.10.4.1 Developmental toxicity in rats

Mefentrifluconazole was administered to groups of 25 presumed-pregnant rats by gavage at dose levels of 0, 50, 150 and 400 mg/kg bw/d during days 6 to 19 of gestation. At terminal sacrifice on gestation day (GD) 20, 24 to 25 females per group had implantation sites.

Only pregnant dams were used for the calculations of mean maternal food consumption, body weight and body-weight change. Only pregnant dams with scheduled sacrifice on GD 20 were taken for the calculation of mean gravid uterine weights, body-weight change corrected for uterine weight and summary of reproduction data. Therefore, the following females were excluded from the above-mentioned calculations: one control female (not pregnant), one mid-dose female (not pregnant) and one high-dose female (not pregnant).

There were no deaths or clinical signs of toxicity in any group. The mean food consumption of the dams treated with 400 mg/kg bw/d was substantially reduced from GD 8 onwards until scheduled sacrifice on GD 20. The average reduction of food consumption in the high-dose dams compared with the controls during the entire treatment period (GD 6-19) was 8 %. Consequently, a treatment-related effect on body weight occurred at 400 mg/kg bw/d from GD 15 onwards. At this dose the mean body-weight gain was statistically significantly reduced during GD 10-15 (up to 27 % below the concurrent control value). When calculated for the entire treatment phase (GD 6-19), the mean body-weight gain was about 17 % below the controls. The mean food consumption, body weights and body-weight gains were unaffected at 50 and 150 mg/kg bw/d.

At sacrifice, the uterus weight, carcass weight and corrected body-weight gain were determined. The mean weights of the unopened uteri were comparable between groups. The corrected body weight gain (terminal body weight on GD 20 minus weight of the unopened uterus minus body weight on GD 6) was statistically significantly lower at 400 mg/kg bw/d (about 34 % below the concurrent control value), as was the carcass weight of the dams of this group (about 7% below controls). Statistically significant changes were not recorded at 50 and 150 mg/kg bw/d.

The caesarean section data showed that 24, 25, 24 and 24 rats were pregnant at 0, 50, 150 and 400 mg/kg bw/d. None of the pregnant dams aborted or gave birth prematurely. One dam of the low-dose group had only resorptions with no live fetuses; otherwise, all dams had viable fetuses. 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 or viable fetuses. Furthermore, all values were within the range of the historical control data performed at the same test facility with the same rat strain (see table below). Exposure to mefentrifluconazole did not result in changes in the sex ratio.

Table 37: Caesarean section data – mefentrifluconazole administration to rats

Dose (mg/kg bw/d)		**0**	**50**	**150**	**400**
Corpora lutea	[N]	10.2 ± 1.50	10.8 ± 2.42	11.1 ± 1.48	11.3 ± 1.52
total number	[N]	245	270	267	272
Implantation sites	[N]	9.7 ± 2.01	9.3 ± 2.65	10.4 ± 1.88	9.9 ± 2.10
total number	[%]	232	232	249	238
Pre-implantation loss	[%]	6.0 ± 11.3	14.7 ± 19.5	7.1 ± 8.7	12.1 ± 17.8
Post-implantation loss	[%]	3.7 ± 6.2	8.2 ± 20.5	7.2 ± 8.0	6.8 ± 9.1
Resorptions	[N]	0.4 ± 0.65	0.4 ± 0.71	0.8 ± 0.88	0.7 ± 0.92
total number	[N]	9	11	19	16
Early resorptions	[N]	0.4 ± 0.65	0.4 ± 0.71	0.8 ± 0.90	0.5 ± 0.59
total number	[N]	9	11	18	11
Late resorptions	[N]	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.20	0.2 ± 0.59
total number	[N]	0	0	1	5
Dead fetuses	[N]	0	0	0	0
Live fetuses	[N]	9.3 ± 1.99	9.2 ± 2.06	9.6 ± 1.74	9.3 ± 2.29
total number	[N]	223	221	230	222
males	[N]	112	109	125	108
females	[%]	111	112	105	114
male / female ratio		50.2 / 49.8	49.3 / 50.7	54.3 / 45.7	48.6 / 51.4
Placental weight	[g]	0.44 ± 0.06	0.44 ± 0.05	0.46 ± 0.05	**0.50** ± 0.07**
males	[g]	0.46 ± 0.07	0.46 ± 0.06	0.47 ± 0.05	**0.51* ± 0.08**
females	[g]	0.43 ± 0.06	0.42 ± 0.05	0.45 ± 0.06	**0.49** ± 0.06**
Fetal weight	[g]	3.6 ± 0.35	3.7 ± 0.13	3.7 ± 0.22	3.5 ± 0.21
males	[g]	3.7 ± 0.36	3.8 ± 0.17	3.8 ± 0.23	3.6 ± 0.22
females	[g]	3.6 ± 0.39	3.5 ± 0.20	3.6 ± 0.22	**3.4* ± 0.24**

* $p < 0.05$, ** $p < 0.01$ (Dunnett test, two-sided)

The mean placenta weights were slightly but statistically significantly increased at 400 mg/kg bw/d (approximately 113 % of the control group value). This change was not associated with impaired fetal development and was, moreover, well within the historical control range and thus is considered by the dossier submitter to be non-adverse. The mean fetal weights were not affected by exposure to mefentrifluconazole at any dose, with the exception of a marginal decrease in the females at 400 mg/kg bw/d (3.4 vs. 3.6 g, 94% of control value). However, as the group mean exactly matches the mean of the historical control (3.4 g) and as there is no effect in the corresponding male fetuses, the dossier submitter concludes that this does not represent a treatment-related effect.

Table 38: Historical control data (rats) – caesarean section data

Historical control data	Mean ± SD	Range (per study) Minimum	Range (per study) Maximum	95% spread 2.5% – 97.5%
Corpora lutea	11.8 ± 1.96	10.0	16.0	n.d.
Implantation sites	11.0 ± 2.26	9.1	15.3	n.d.
Pre-implantation loss [mean %]	6.9 ± 13.09	1.4	17.5	n.d.
Post-implantation loss [mean %]	7.3 ± 11.19	3.5	18.1	n.d.
Resorptions [N]	0.8 ± 1.00	0.3	1.5	n.d.
Live litter size [N]	10.2 ± 2.34	8.3	14.8	n.d.
Placenta weights [g]	0.46	0.33	1.16	0.32 – 0.60
Males	0.47	0.30	1.16	0.33 – 0.61
Females	0.45	0.28	0.99	0.32 – 0.58
Fetal weights [g]	3.5	2.3	5.1	2.2 – 4.1
Males	3.6	2.4	5.4	3.0 – 4.2
Females	3.4	2.2	4.9	2.9 – 4.1

76 studies performed at the test facility between 2009–2014 with Wistar rats (Charles River)
(1518 pregnant dams; 1502 litters with 15402 viable fetuses)

External malformations were recorded in 0, 0, 1 (mandibular micrognathia with severely malformed skull bones) and 1 (multiple malformations: cleft palate, microphthalmia, malformed mandible) fetuses at 0, 50, 150 and 400 mg/kg bw/d. Historical control data from the same laboratory and rat strain showed that multiple malformations of the skull bones appeared fairly frequently and uniformly between 2009 and 2016 (76 studies between 2009 and 2014: 10 cases, range of fetal incidences 0 to 2 %; 79 studies between 2011 and 2016: 11 cases, range of fetal incidences 0 to 2 %). External variations were recorded in 0, 1 (limb hyperflexion), 0 and 0 fetuses at 0, 50, 150 and 400 mg/kg bw/d. There was therefore no association of external malformations and variations with exposure to mefentrifluconazole.

Upon visceral examination, two soft tissue variations were noted in both the control and exposed groups but without statistical significance. Dilated renal pelvis was noted in 2 (1.9 %), 1 (0.9 %), 3 (2.7 %) and 8 (7.6 %) fetuses at 0, 50, 150 and 400 mg/kg bw/d. The litter incidence for this variation was 2 (8.3 %), 1 (4.2 %), 1 (13 %) and 5 (21 %), respectively. In historical control data from 75 studies performed at the test facility between 2009 and 2014 with Wistar rats, the range for the fetal incidence was 0-11.8 % (mean 2.5 %), whilst the range for the litter incidence was 0-57.1 % (mean 11.9 %) (1453 litters with 7096 viable fetuses examined). Dilated ureter was observed in one fetus from each of the control and high-dose groups. Therefore, there was no treatment-related effect on the incidence of visceral malformations or variations in this study.

Skeletal malformations were noted in 2, 0, 1 and 2 fetuses at 0, 50, 150 and 400 mg/kg bw/d. These consisted of misshapen cervical vertebra (control), severely malformed sternum (control), severely malformed skull bones (mid-dose group), malpositioned and bipartite sternebra (high-dose group) and multiple skeletal malformations (high-dose group; also described in section on external findings). There was therefore no relationship of these findings to exposure to mefentrifluconazole.

Statistically significant increases in the incidence of two skeletal variations were recorded, comprising an increase in supra-occipital holes at 150 and 400 mg/kg bw/d (but without a clear dose-response relationship) and an increase in misshapen sacral vertebrae at 400mg/kg bw/d. The historical control data provided by the applicant, from the same test facility and appropriate time-span and in Wistar rats, is presented below. The fetal incidences, litter incidences and mean affected fetuses / litter were within the historical control ranges. A dose-related increase in the number of fetuses with dumb-bell ossification of the thoracic centrum with dumb-bell-shaped cartilage of the centrum was noted, but none of the increases was statistically significant at any dose and all the incidences were within the historical control data; this finding cannot, therefore, be positively attributed to mefentrifluconazole.

Table 39: Skeletal variations in rats exposed to mefentrifluconazole (selected)

Dose (mg/kg bw/d)		**0**	**50**	**150**	**400**
Litters evaluated		24	24	24	24
Fetuses evaluated		117	115	120	117
Live		117	115	120	117
Dead		0	0	0	0
Supra-occipital hole(s)					
Fetal incidence	# (%)	8 (6.8)	6 (5.2)	21 (18)	17 (15)
Litter incidence	# (%)	6 (25)	5 (21)	**15 (63)****	**13 (54)***
Affected fetuses / litter	%	6.5 ± 12.18	5.1 ± 10.81	**18.4 ± 19.24****	**17.9 ± 23.44***
Misshapen sacral vertebra					
Fetal incidence	# (%)	3 (2.6)	5 (4.3)	7 (5.8)	12 (10)
Litter incidence	# (%)	3 (15)	3 (13)	5 (21)	**9 (38)***
Affected fetuses / litter	%	2.7 ± 7.37	3.9 ± 10.79	5.8 ± 12.55	**10.9 ± 18.06***
Dumb-bell ossification of thoracic centrum with dumb-bell-shaped cartilage of centrum					
Fetal incidence	# (%)	7 (6.0)	8 (7.0)	9 (7.5)	18 (15)
Litter incidence	# (%)	6 (25)	7 (29)	7 (29)	10 (42)
Affected fetuses / litter	%	5.6 ± 10.83	7.1 ± 11.90	7.8 ± 14.82	14.2 ± 20.63

Statistics, litter incidence: Fisher's Exact Test (1-sided); affected fetuses/litter: Wilcoxon-Test (1-sided)
* : $p \leq 0.05$; ** : $p \leq 0.01$

Table 40: Historical control data – fetal skeletal variations (rats)

	Fetuses (7684)			**Litters (1431)**			**Affected fetuses / litter**	
	No.	%	Range	No.	%	Range	% Mean	Range
Supra-occipital holes	954	12.4	0.0 – 52.3	521	36.4	0.0 – 100.0	12.2	0.0 – 50.8
Misshapen sacral vertebra	250	3.3	0.0 – 10.2	212	14.8	0.0 – 41.7	3.4	0.0 – 10.9
Dumb-bell ossification of thoracic centrum with dumb-bell-shaped cartilage of centrum	318	4.1	0.0 – 16.2	249	17.4	0.0 – 56.0	4.3	0.0 – 19.4

76 studies performed at the test facility (2009–2014) with Wistar rats (Charles River) (1431 litters with 7684 viable fetuses examined)

The laboratory use of the nomenclature 'misshapen sacral vertebra' refers to a minor change in the direction (from ventral to cranial, i.e. either to the left or right) of one of the sacral vertebral arches (generally the first one), thus giving the first sacral vertebra a more 'lumbar-like' appearance. Cartilage was also reported to be present. In the study authors' experience, this is a minor anatomic variant that is neither permanent nor detrimental to post-natal survival or health. It is thus appropriately classified as a small anatomical variation. Dumb-bell shaped ossification of the vertebral centrum is also classified as a variation of low concern. Supra-occipital holes are very small, discrete areas with no ossification or bone precursor and, as shown by the historical control data, are very common spontaneous findings. These represent a slight developmental delay without any adverse effects on development and thus do not provide evidence of developmental toxicity.

Overall, there was no relationship to mefentrifluconazole exposure in the incidence of total external, visceral and skeletal observations (see table below).

Table 41: Total malformations and variations – rat study

Dose (mg/kg bw/d)		**0**	**50**	**150**	**400**
Litters evaluated		24	24	24	24
Fetuses evaluated		223	221	230	222
Live		223	221	230	222
Dead		0	0	0	0
Total malformations					
Fetal incidence	# (%)	3 (1.3)	0 (0.0)	1 (0.4)	2 (0.9)
Litter incidence	# (%)	3 (13)	0 (0.0)	1 (4.2)	2 (8.3)
Affected fetuses / litter	%	1.3 ± 3.41	0.0 ± 0.00	0.5 ± 2.27	1.0 ± 3.68
Total variations					
Fetal incidence	# (%)	119 (53)	116 (52)	123 (53)	124 (56)
Litter incidence	# (%)	24 (100)	24 (100)	24 (100)	24 (100)
Affected fetuses / litter	%	53.5 ± 4.49	52.7 ± 3.52	53.5 ± 3.66	55.7 ± 8.13

Statistics, litter incidence: Fisher's Exact Test (1-sided); affected fetuses/litter: Wilcoxon-Test (1-sided)
* $p < 0.05$, ** $p < 0.01$

10.10.4.2 Developmental toxicity in rabbits

An oral (gavage) developmental toxicity study has been conducted in rabbits at doses of 0, 5, 10 and 25 mg/kg bw/d (30-33 presumed-pregnant females / group). The doses were selected on the basis of three range-finding studies. In the first, doses of 50, 150 and 400 mg/kg bw/d were tested in three female non-pregnant New Zealand White rabbits per group; 2 / 3 does exposed to 50 mg/kg bw/d were sacrificed in a moribund condition, with signs of poor or reduced nutritional condition, no or reduced faeces (3 / 3 animals) and lateral position (1 / 3 animals). At 150 and 400 mg/kg bw/d, all animals either died or were sacrificed because of poor condition, with signs that were similar to those at the low-dose but that occurred earlier and were more severe. At all doses, food consumption was reduced to almost zero levels within 2-5 days of the commencement of the study. As a consequence, the animals constantly lost weight until their pre-terminal death or sacrifice. For these reasons, the dose level of 50 mg/kg bw/d was considered to be potentially lethal. In the second range-finding study, similar signs of toxicity (reduced food and water consumption, reduced faeces, body-weight loss) occurred in 1 / 3 females at 25 mg/kg bw/d, resulting in sacrifice of this animal on day 17. At necropsy, the small intestine, large intestine and rectum were empty of contents. All parameters in the remaining two animals were equivalent to the controls. Subsequently, groups of five pregnant New Zealand White rabbits were administered the test substance by oral gavage at doses of 0, 5, 10 and 20 mg/kg bw/d from gestation days 6 to 28; there were no consistent adverse effects at any dose. The chosen highest dose for the main study, 25 mg/kg bw/d, represented half the lethal dose in non-pregnant animals.

In the main study, animals were dosed from GD 6 to 28. At sacrifice on GD 29, 20 to 24 females per group had implantation sites. Only pregnant does were used for the calculations of mean maternal food consumption, body weight and body-weight change. Only pregnant does with scheduled sacrifice on GD 29 were taken for the calculation of mean gravid uterine weights, corrected body weight gain and summary of reproduction data. In accordance with the test guideline, each group contained at least 16 females with implantation sites at the time of necropsy.

One low-dose female and one mid-dose female were sacrificed after they had spontaneous abortions. One female from each of the control and high-dose groups was found dead following gavage errors. Otherwise, there were no deaths. There were no overt clinical signs of toxicity.

Food consumption of the high-dose group was transiently decreased (87 % of controls) on gestation days 6-7 and in the mid-dose group it was transiently increased (126 % of controls) on GD 22-23. Overall, however, the values were comparable in all the groups (days 0-29: 100 %, 106 % and 96 % of control values at 5, 15 and 25 mg/kg bw/d). The mean body weights and body-weight change were also unaffected by exposure to mefentrifluconazole (body-weight change days 0-29: 91 %, 100 %, 87 % at 5, 15 and 25 mg/kg bw/d, not statistically significant).

Clinical pathology did not reveal any changes in haematology parameters. ALT and AST activities were decreased at 25 mg/kg bw/d; AST activity was also lower at 15 mg/kg bw/d. Since the decreases were slight (ALT by 24%; AST by 34% at 25 mg/kg bw/d) and were not accompanied by other changed liver parameters, the dossier submitter concludes they were treatment-related but not adverse. The globulin level was lower in the high-dose rabbits but this, again, was likely to be adaptive rather than adverse, since no other changed clinical-chemistry parameters were noted.

At scheduled sacrifice, the mean carcass weights and corrected body weight (terminal body weight on GD 29 minus weight of the unopened uterus minus body weight on GD 6) were comparable between all the groups. The mean gravid uterus weights of the dosed rabbits were not influenced by the test substance. There were no treatment-related observations upon gross necropsy.

The caesarean-section data showed that at least 20 pregnant females were available in each group. The number of females that were pregnant at terminal sacrifice was slightly reduced at 5 and 15 mg/kg bw/d compared with the control and high-dose groups, because of several animals that weren't pregnant and one in each of these (low- and mid-dose) groups that aborted; since there wasn't a dose-response relationship, the dossier submitter concludes that this wasn't a response to mefentrifluconazole exposure. There were no treatment-related effects on the number of pre- and post-implantation losses or number of resorptions. At 15 mg/kg bw/d, the number of implantation sites (mean of 7.0 per dam) and subsequently the live-litter size (mean of 6.4 per dam) was statistically significantly lower than the controls and slightly below the historical control range. These lower values were the consequence of a mean number of corpora lutea that was below the historical control range and a higher pre-implantation loss (within the historical control range) and hence were unrelated to mefentrifluconazole exposure. Furthermore, there was no dose-response relationship in the number of implantation sites and live-litter size, and no adverse findings in the reproductive organs of the affected animals. All other differences observed reflected the normal range of fluctuations for animals of this strain and age; this included a higher post-implantation loss in the control and 15 mg/kg bw/d groups, which were caused by single does in each group with spontaneously-resorbed litters. The mean number and weight of live fetuses, male and female fetuses, the sex ratio and placental weights were not affected by treatment.

Table 42: Pregnancy status and caesarean section data

Dose (mg/kg bw/d)		**0**	**5**	**15**	**25**
Pregnancy status					
Females					
- mated	[n]	27	30	33	30
- pregnant	[n]	25	21	23	23
conception rate	[%]	93	70	70	77
- aborted	[n]	0	1	1	0
- premature birth	[n]	0	0	0	0
- Does with viable fetuses	[n]	23	20	21	22
- Does with all resorptions	[n]	1	0	1	0
- Death		1	1	1	1
- Pregnant terminal sacrifice	[n]	24	**20***	**22***	22
Caesarean section data[a]					
- Corpora lutea	[n]	9.4 ± 2.67	8.9 ± 2.35	8.1 ± 2.41	9.1 ± 1.96
total number	[n]	225	179	178	201
- Implantation sites	[n]	8.9 ± 2.61	8.4 ± 2.54	**7.0*** ± 2.80	8.4 ± 2.52
total number	[n]	213	169	153	184
- Pre-implantation loss	[%]	4.8 ± 10.59	6.3 ± 9.99	14.5 ± 21.13	10.4 ± 16.18
- Post-implantation loss	[%]	12.6 ± 21.23	4.7 ± 7.46	12.4 ± 24.90	3.7 ± 6.25
- Resorptions	[n]	0.8 ± 0.78	0.4 ± 0.60	0.8 ± 1.97	0.4 ± 0.66
total number	[n]	19	8	18	8
- Early resorptions	[%]	10.8 ± 21.33	2.9 ± 6.65	11.4 ± 25.00	2.0 ± 4.45
number	[n]	0.6 ± 0.71	0.2 ± 0.41	0.7 ± 1.96	0.2 ± 0.39
total number	[n]	15	4	16	4
- Late resorptions	[%]	1.8 ± 5.62	1.8 ± 4.73	1.0 ± 3.22	1.6 ± 4.43
number	[n]	0.2 ± 0.48	0.2 ± 0.52	0.1 ± 0.29	0.2 ± 0.50
total number	[n]	4	4	2	4
- Dead fetuses	[n]	0	0	0	0
- Does with viable fetuses	[n]	23	20	21	22
- Live fetuses		8.4 ± 2.29	8.1 ± 2.42	**6.4*** ± 2.93	8.0 ± 2.31
total number	[n]	194	161	135	176
Mean	[%]	91.2 ± 10.42	95.3 ± 7.46	91.8 ± 15.77	96.3 ± 6.25
- Total live female fetuses	[n]	4.5 ± 1.83	4.4 ± 1.82	**3.0*** ± 1.75	3.8 ± 1.71
total number	[n]	104	89	64	84
Mean	[%]	49.5 ± 18.05	50.9 ± 18.24	42.7 ± 19.99	47.5 ± 20.13
- Total live male fetuses	[n]	3.9 ± 1.81	3.6 ± 1.31	3.4 ± 1.91	4.2 ± 1.87
total number	[n]	90	72	71	92
Mean	[%]	41.7 ± 14.49	44.4 ± 16.24	49.1 ± 21.89	48.8 ± 18.59
- Percent live females		53.6	55.3	47.4	47.7
- Percent live males		46.4	44.7	52.6	52.3
Placental weights	[g]	5.5 ± 0.81	5.5 ± 0.89	5.6 ± 0.93	5.3 ± 0.75
- male fetuses	[g]	5.5 ± 0.99	5.6 ± 0.94	5.6 ± 0.88	5.2 ± 0.56
- female fetuses	[g]	5.4 ± 0.81	5.4 ± 0.81	5.4 ± 0.91	5.2 ± 0.82
Mean fetal weight	[g]	39.4 ± 6.25	40.6 ± 5.00	42.6 ± 5.19	39.8 ± 6.55
- males	[g]	39.6 ± 7.32	39.9 ± 5.29	42.3 ± 5.03	39.9 ± 5.50
- females	[g]	38.7 ± 6.39	40.0 ± 4.84	41.9 ± 5.25	39.0 ± 6.97

[a] Mean ± SD on litter basis; Statistical evaluation: * $p \leq 0.05$; ** $p < 0.01$ (Dunnett-test, two-sided)

Table 43: Historical control data (rabbits) – caesarean section data

Historical control data	Mean	±	SD	Range (per study)	
				Minimum	Maximum
Corpora lutea	9.9	±	2.56	9.0	11.0
Implantation sites	8.8	±	2.98	7.1	10.3
Pre-implantation loss [mean %]	11.9	±	18.66	5.4	25.7
Post-implantation loss [mean %]	6.9	±	13.07	2.4	11.3
Resorptions [N]	0.6	±	0.99	0.3	1.1
Live litter size [N]	8.2	±	2.90	6.6	9.7

12 studies performed at test facility (Jan 2009– Sep 2013) with New Zealand White rabbits (Charles River); (303 pregnant dams; 285 litters with 2366 viable fetuses)

There were no treatment-related external malformations (fetal incidence: 2, 0, 0 and 0 at 0, 5, 15 and 25 mg/kg bw/d), external variations (1, 0, 0, 0 fetuses in the respective groups) or unclassified external findings (findings that could not be attributed to either the malformation or variation classifications).

There were no treatment-related visceral malformations (1 in each of the groups), visceral variations (fetal incidence: 4, 5, 2, 3 at 0, 5, 15 and 25 mg/kg bw/d) nor soft-tissue unclassified findings (2, 1, 1, 1 fetuses in the respective groups). The visceral malformations comprised absent subclavian at 0, 5, 25 mg/kg bw/d, together with small thymus in the high-dose group and diaphragmatic hernia at 15 mg/kg bw/d. The soft tissue variations included dilated cerebral ventricle (1 fetus at 25 mg/kg bw/d), cystic dilatation in the brain (1 fetus each at 5 and 15 mg/kg bw/d), malpositioned carotid branch (1 fetus in each of 0, 5, 25 mg/kg bw/d groups, 2 at 15 mg/kg bw/d), short innominate (1 fetus at 25 mg/kg bw/d) and absent lung lobe (3 control fetuses, 2 at 5 mg/kg bw/d).

Skeletal malformations were recorded in 7, 2, 1, and 3 fetuses at 0, 5, 15 and 25 mg/kg bw/d. The malformations observed in the high-dose group comprised one fetus with misshapen interparietal (compared with 2 controls) and two with a severely malformed sternum (3 in the controls); one of these high-dose fetuses also had absent subclavian and small thymus. Skeletal variations of different bone structures were observed, with or without effects on the corresponding cartilage, in all groups without a dose-response relationship; variations occurred in almost all animals, which is explained by the common finding of incomplete ossification: of the hyoid (fetal incidence = 43 % of controls, 40 % at 25 mg/kg bw/d), the cervical centrum (fetal incidence = 16 % of controls, 11 % at 25 mg/kg bw/d) and the sternebrae (45 % of control fetuses, 28 % at 25 mg/kg bw/d). One finding that was classified by the study authors as a skeletal variation (fused sternebra with unchanged cartilage) occurred in a higher incidence in the high-dose group than the controls (see table below), although without statistical significance; the incidences were above the historical control ranges from the test facility. The dossier submitter notes, however, that the litter incidence represented only one litter above the historical-control range (18 % = 4/22 litters in the high-dose group, compared with 14.3 % = 3/21 in the historical-control data). There were no treatment-related unclassified cartilage observations.

Table 44: Skeletal variations (selected) in rabbit study

Dose (mg/kg bw/d)		0	5	15	25
Litters evaluated		23	20	21	22
Fetuses evaluated		194	161	135	176
Live		194	161	135	176
Dead		0	0	0	0
Fused sternebra; unchanged cartilage					
Fetal incidence	# (%)	1 (0.5)	3 (1.9)	1 (0.7)	7 (4.0)
Litter incidence	# (%)	1 (4.3)	2 (10)	1 (4.8)	4 (18)
Affected fetuses / litter	%	0.5 ± 2.32	3.3 ± 10.26	0.5 ± 2.42	5.5 ± 15.4

Statistics, litter incidence: Fisher's Exact Test (1-sided); affected fetuses/litter: Wilcoxon-Test (1-sided)
* : $p \leq 0.05$; ** : $p \leq 0.01$

Table 45: Historical control data – fetal skeletal variations (rabbits)

	Fetuses (2894)			Litters (349)			Affected fetuses / litter	
	No.	%	Range	No.	%	Range	% Mean	Range
Fused sternebra; unchanged cartilage	25	0.9	0.0 – 1.9	22	6.3	0.0 – 14.3	0.8	0.0 – 2.1

12 studies performed at the test facility (2009–2013) with New Zealand White rabbits (Charles River) (349 litters with 2894 viable fetuses examined)

Three of the fetuses in the high-dose group that were diagnosed with fused sternebrae were in the same litter (number 80). Double-staining for bone and cartilage enabled an assessment of the magnitude of the morphologic change. The sternebral fusion of all these fetuses showed the same characteristics: two sternebrae (either the 3rd and 4th, or the 4th and 5th) linked by a weak bone bridge and, additionally, being misshapen. The litter size was 8 fetuses, so that 38 % of the litter had this variation. Dam number 80 showed no overt clinical signs of toxicity, but its carcass weight was 16 % below that of the mean of the high-dose group and 17 % below the mean control value; moreover, its body weight on day 0 (before the start of exposure) was 10 % lower than the group mean, such that it was one of the dams with the lowest body weight at the start of the study in any of the groups. Therefore, this variation might have resulted from a slight developmental delay, resulting from the lower maternal body weight of this dam. Across all the groups, many of the affected fetuses were amongst the smallest in their respective litters, which would support the finding being representative of a minor delay. The other four affected fetuses in the high-dose group (from three different litters) showed the same morphological characteristics, as did all the affected fetuses in the control, low- and mid-dose groups and controls from the historical control studies. The fusions were of minimal magnitude and confined to individual sternal embryonic areas rather than affecting the whole sternum; additionally, the pattern of sternal changes was identical between the affected control animals and those treated with mefentrifluconazole. In all cases, the underlying cartilage was normal without any indication of a change. These characteristics indicate negligible consequences for post-natal development. In particular, the presence of normal underlying cartilage provides evidence of a simple delay or minor disturbance of ossification that is likely to disappear post-natally.

Skeletal examination of fetuses in developmental toxicity studies represents a single ‘snapshot’ in time; hence, an appreciation of the sequence and normal patterns of ossification aids in the differentiation of generalised delays and minor alterations from true skeletal dysplasia. In rodents and rabbits, the sternebrae are amongst the regions that ossify rapidly during late gestation: sternebrae 1 to 4 ossify first, followed by sternebra 6, with sternebra 5 being last. Variable ossification of these late-ossifying bones is normal in rodents and rabbits, with the incidence of fetuses with ossification in these sites being dependent upon the day of gestation at sacrifice and the criteria used by each laboratory for individual bones. In laboratories that perform Caesarean section on GD 29, as was the case in the present study, alterations of sternal elements (unossifications, misalignments, fusions, misshapes, attachments) are amongst the most commonly occurring developmental variations in New Zealand White rabbits.

The dossier submitter therefore considers that this finding represents a marginal variation that has no adverse consequences and would disappear over time. Taken together with the lack of a dose-response relationship and statistical significance for the findings, the dossier submitter concludes that the slightly higher incidence of this variation in the high-dose group does not constitute evidence of a developmental effect. Furthermore, mefentrifluconazole clearly did not induce malformations of the sternum, since there were more cases of a malformed sternum in the controls than in the high-dose group.

Overall, exposure to mefentrifluconazole did not result in increased total fetal or litter incidences of malformations or variations in rabbits.

Table 46: Total malformations and variations – rabbit study

Dose (mg/kg bw/d)		**0**	**50**	**150**	**400**
Litters evaluated		23	20	21	22
Fetuses evaluated		194	161	135	176
Total fetal malformations					
Fetal incidence	# (%)	8 (4.1)	3 (1.9)	2 (1.5)	3 (1.7)
Litter incidence	# (%)	6 (26)	3 (15)	2 (9.5)	3 (14)
Affected fetuses / litter	%	3.8	2.1	1.4	1.7
Total fetal variations					
Fetal incidence	# (%)	191 (98)	160 (99)	134 (99)	173 (98)
Litter incidence	# (%)	23 (100)	20 (100)	21 (100)	22 (100)
Affected fetuses / litter	%	98.7	99.4	99.5	98.4

Statistics, litter incidence: Fisher's Exact Test (1-sided); affected fetuses/litter: Wilcoxon-Test (1-sided)
* $p < 0.05$, ** $p < 0.01$

10.10.5 Short summary and overall relevance of the provided information on adverse effects on development

The developmental toxicity of mefentrifluconazole has been investigated in rats and 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 fetuses. There was also no consistent evidence that the test substance affected fetal weights.

Two fetuses had multiple malformations, one in the mid-dose group and one in the high-dose group. The malformations in each fetus 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.

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, mefentrifluconazole was not a developmental toxicant in rats or rabbits under the conditions of the available studies.

10.10.6 Comparison with the CLP criteria

A classification for developmental toxicity might be warranted where there is evidence from humans or experimental animals of an adverse effect on the development of the conceptus either before or after birth. Such effects shall have been observed in the absence of other toxic effects, or shall not be secondary non-specific consequences of the other toxic effects.

No information is available in humans.

In a developmental toxicity study in rats, doses of 400 mg/kg bw/d resulted in maternal toxicity, which manifested as reduced food consumption, body weight and body-weight gain. At this dose, there was no evidence of intra-uterine toxicity to the embryos / fetuses or an induction of malformations. All skeletal variations were within the historical control ranges. This study thus did not demonstrate any developmental toxicity potential of mefentrifluconazole in rats.

Doses up to 25 mg/kg bw/d have been investigated in an oral developmental toxicity study in rabbits. This dose did not induce evident maternal toxicity (no overt clinical signs; food consumption, body weight, body-weight gain and carcass weights were unaffected). There was no evidence of intra-uterine toxicity, nor were there statistically significant or dose-related increases in any type of malformation, variation, or unclassified observation.

In conclusion, mefentrifluconazole did not meet the criteria for classification for developmental toxicity in these studies.

10.10.7 Adverse effects on or via lactation

Information on the potential for mefentrifluconazole to induce adverse effects on or via lactation is provided by a two-generation study in rats (described in section 10.10.1).

In this study, slight reductions in the viability index in the high-dose group (200 mg/kg bw/d) of each generation (93.5 % and 93.6 % in the F_1 and F_2 generations, respectively, compared with 99.1 % and 99.7 % in the controls), which were not statistically significant, were a consequence of single whole-litter losses; these appeared to be the result of inadequate nursing, arising from the much reduced food intake of the respective dams. The overall lower food consumption and lower body weights early in lactation of the dams in the high-dose group affected development of the pups: in each generation, pup body weights of the 200 mg/kg bw/d group were reduced compared with the controls from post-natal day 1 and throughout lactation. Notwithstanding, pup survival between lactation days 4 and 21 was high (> 99.4 %) in all the groups of each generation.

The dossier submitter concludes that the observed effects on pups were secondary to maternal toxicity and not a direct toxic effect of mefentrifluconazole or its metabolites on or via lactation.

10.10.8 Short summary and overall relevance of the provided information on effects on or via lactation

See section 10.10.7.

10.10.9 Comparison with the CLP criteria

Classification for effects on or via lactation might be assigned where: there is human evidence that indicates a hazard to babies during the lactation period; the results of one- or two-generation studies in animals provide clear evidence of adverse effects in the offspring owing to transfer in the milk or adverse effect on the quality of the milk; toxicokinetic studies indicate the likelihood that the substance is present in potentially toxic levels in breast milk.

In the case of mefentrifluconazole, there is no human data to inform on this end-point, nor is there information from toxicokinetic studies to indicate that the substance would be present in breast milk at potentially toxic levels.

The available two-generation study in rats indicates that there are no effects on offspring that can be attributed to mefentrifluconazole or its metabolites in milk. The observed slight reduction in early post-natal survival can be attributed to maternal toxicity. The mothers of the affected litters showed markedly reduced feed intake during lactation with associated signs of insufficient maternal care, leading to a slight increase in pup mortality and decreased body weights in the surviving pups.

Mefentrifluconazole, therefore, does not meet the criteria for classification for effects on or via lactation.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

Not classified (conclusive but not sufficient for 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.

Supplemental information - In depth analyses by *RAC*

Parental toxicity

No treatment-related death and/or clinical signs were recorded in any group. The doses of 25 and 75 mg/kg bw/day did not produce modifications when compared with the concurrent control in terms of body weight, body weight gain and food consumption. Also, there were no treatment-related findings in either gross pathology or histopathology. However, the dose of 75 mg/kg bw/day induced slight liver dysfunction evidenced as increased ALP (both males and females), cholesterol (males) and liver weight (males and females).

The high dose of 200 mg/kg bw/day induced the following signs of toxicity:

a) F_0 generation
    1. decreased body weight, body-weight gain and food consumption in both males and females. In F_0 males body weights were statistically significantly below the concurrent controls starting from pre-mating day 13; up to 11% reduction was recorded at the end of the study. The body-weight gain of these males was statistically significantly below the control values in the pre-mating period (up to 33%) and consistently lower (but without statistical significance) during other study periods (mating, post-mating). In F_0 females, body weights were consistently below concurrent controls throughout the study, and gained statistical significance during pre-mating days 48–55 (up to 5% reduction), during the entire gestation period (up to 9%) and during lactation days 1–14 (up to 13% decrease). The body-weight gain of these females was statistically significantly below the control values during key parts of the gestation period (up to 24%), although they generally gained more weight during lactation (23.7 g, 26.6 g, 34.5 g. 44.9 g at 0, 25, 75, 200 mg/kg bw/day, gain over lactation day 1 to 21);
    2. increased liver cell hypertrophy in 15 out of 25 males only;
    3. increased cholesterol in males only;
    4. one dam with total litter loss by post-natal day number two (PND 2); one pup from this litter showed signs of insufficient nursing (no milk in the stomach). No findings were found in the 5 other pups; the rest of the litter was cannibalised and could not be assesed.

b) F_1 generation
    1. decreased body weight, body-weight gain and food consumption in both males and females. The body weights of the F_1 males were statistically significantly below the controls from the beginning of pre-mating onwards and remained so until the end of the study (up to 12 % decrease). The body weight gain of these males was statistically significantly below the control values during key parts of

the pre-mating period (up to 32 % lower) and during post-mating days 0-7 (approx. 58 %). The body weights of the F_1 females were statistically significantly below the controls during the entire pre-mating, gestation and lactation periods (decreases of up to 11 %, 16 % and 17 %, respectively). The body-weight gain of these females was below the controls during most of the pre-mating period (days 0–69, reduced by about 11%) and throughout gestation (up to 32 % lower). In contrast, these females generally gained more weight during lactation 1-21 days (28.2, 26.4, 29.9 and 36.5 g at 0, 25, 75 and 200 mg/kg bw/day). One high-dose F_1 female showed severe effects including poor general condition, piloerection and inability to deliver on gestation day 23, followed by delivery of a still-born litter on GD24;

2. increased ALP (males and females), urea, inorganic phosphate (males) and triglycerides (females);
3. increased liver weight (both males and females) and liver cell hypertrophy in males only (15 out of 25 – same as in the F_0 generation);
4. one dam with total litter loss by PND 3; pups showed signs of insufficient nursing (reduced nutritional condition, no milk in the stomach);
5. one dam with only stillborn pups; this dam showed poor general state and piloerection.

It is noted that the majority of the relative organ weight (adjusted for body weight) values were not different from the controls. The only exception is the increase in the liver weights of both males and females at 75 and 200 mg/kg bw/day in both generations.

The regular oestrus cycles were observed in all groups of all generations. However, the value for the F_1 high-dose females was statistically significantly ($p < 0.05$) longer than the control value by half a day. As a result, the number of cycles within the observation period was lower (3.48 versus 4.32 in controls, $p \leq 0.01$). Although statistically significant, the longer duration of the cycles was within the HCD of the test facility: range = 3.99 – 4.8, mean = 4.33 days. Furthermore, neither time to pairing nor pairing success were affected. Therefore, the DS did not consider this slight lengthening of the oestrus cycle as evidence of an adverse effect.

Male reproductive parameters

The male mating index was 100% in all groups in both generations and so was the male fertility index with one exception: in the F_1 high dose group two males did not generate pups and the corresponding fertility index became 92%. However, this value is within the HCD: range = 80-100%, mean = 91.8%. The two animals were necropsied and in addition to an absence of gross or histopathological findings, no changes in sperm quality that would suggest infertility were found.

In general, sperm analyses, which investigated sperm motility and determined the incidence of sperm head counts in the testes, as well as the percentage of abnormal sperm in the testes and cauda epididymis, did not indicate any effects of treatment in F_0 or F_1 males.

Female reproductive parameters

A summary of data regarding the female reproduction parameters provided by the DS is reproduced in the following table:

Parental generation	**F_0**				**F_1**			
Dose level [mg/kg bw/day]	**0**	**25**	**75**	**200**	**0**	**25**	**75**	**200**

No. of animals per group	25	25	25	25	25	25	25	25
No. of females placed with males	25	25	24	25	25	25	25	25
No. of females mated	25	25	24	25	25	25	25	25
Female mating index [%]	100	100	100	100	100	100	100	100
No. of females pregnant	25	25	24	25	25	25	25	23
Female fertility index [%]	100	100	100	100	100	100	100	92
Pre-coital interval [mean days]	2.8	2.4	3.0	2.8	3.0	3.0	2.5	2.8
Duration of gestation [mean days]	22.1	22.2	22.2	**22.4***	22.2	22.0	22.0	22.2
Implantation sites, total	307	284	288	295	300	285	308	229
- per dam	12.3	11.4	12.0	11.8	12.0	11.4	12.3	**10.0***
Post-implantation loss [mean %]	3.9	5.5	1.3	5.2	2.4	5.0	**7.1****	8.9
Females with live-born	25	25	24	25	25	25	25	21
- with still-born pups	1	1	0	1	1	3	2	3
- with all still-born	0	0	0	0	0	0	0	1
Gestation index [%]	100	100	100	100	100	100	100	91.3
Pups delivered	297	267	284	277	298	269	285	217
- per dam	11.9	**10.7***	11.8	11.1	11.9	10.8	11.4	**9.9****
- live-born	296	266	284	274	295	263	283	208
- still-born	1	1	0	3	3	6	2	9
Live-birth index [%]	99.7	99.6	100	98.9	99.0	97.8	99.3	95.9

Statistical analysis: *: $p \leq 0.05$; **: $p \leq 0.01$

Historical control data [33 studies over 2008-2015 at the test facility with Wistar rats]

- Duration of gestation: 21.8 – 22.9 days
- Gestation index: 87.5 – 100 %
- Implantation sites/dam: 9.4 – 14.0
- Post-implantation loss [mean %]: 0.9 – 17.7
- Pups delivered/dam: 9.2 – 13.4
- Live-birth index [mean %]: 92.1 – 100

In the F_0 generation, exposure to mefentrifluconazole did not affect the mating, fertility or gestation indexes. The mean duration of gestation was slightly increased in the high-dose group (22.4 compared with 22.1 in controls, $p \leq 0.05$). In absolute terms this represented a difference from the control group of less than half a day and the value is within the HCD of the test facility: 21.9 to 22.9 days. The live-birth index was lower in the high dose but comparable with the concurrent controls and within the HCD. The number of pups delivered per dam was slightly lower in the 25 mg/kg bw/day dose but, since no dose-effect relationship was shown, it appears to not have been treatment-related. The post-natal survival rate at the high dose was affected by one dam which delivered eleven live pups on GD23 (none dead) but had total litter loss on post-natal day 2. This dam had almost half the food consumption (16.5 g) compared to the group means (32.0, 30.8, 32.6 and 28.8 g at 0, 25, 75 and 200 mg/kg bw/day, respectively). The mean number of implantation sites was comparable between all groups. The slightly lower number of post-implantation losses at 25 mg/kg bw/day was not statistically significant and, as in the case of the pups delivered per dam, it appears not to have been treatment-related.

In the F_1 generation the female mating index was not affected but the fertility index was lower at the higher dose of 200 mg/kg bw/day. However, the value was within the range of the contemporary historical control data of the test facility in the same strain: 80 to 100 %. The

low percentage was due two females which were sperm-positive but did not become pregnant. The necropsy revealed neither gross nor histopathological changes in the sexual organs of these females. Also, time-to-mating was not different. The gestation index of the high dose group was also slightly lower when compared with the other groups (91.3 vs 100% for all groups). This lower value is due to two females that were pregnant but did not deliver any live pups (one with 6 still-born pups and the other with 1 implantation site, no pups present).The lower gestation index was within the HCD (87.5 to 100 %), where four females had entirely still-born litters.

The mean number of implantation sites per dam was slightly but statistically significantly lower in the high-dose-group (mean 10.0, range 1 to 14, compared with mean 12.0, range 9 to 16 in the controls). However, the value fits within the HCD range (33 studies over 2008-2015 at the test facility with Wistar rats: 9.4 to 14) and no dose-effect relationship was seen. The mean was affected by one female that contained only one implant.

A dose dependent increase of the mean post-implantation losses was observed, which could suggest a dose-related effect. However, all the values were within the HCD (33 studies over 2008-2015 at the test facility with Wistar rats: 0.9 to 17.7). Moreover, only the mid-dose value was statistically significant when compared with the concurrent controls. Finally, the value for the high-dose of 200 mg/kg bw/day is influenced by the same female that influenced the mean number of implantation sites: the only implant seen in this animal was resorbed resulting in 100% loss. Exclusion of this animal from the calculation would lead to a group mean of 4.8 % i.e. consistent with the mean value of the lowest dose group (5.0%).

The mean number of pups delivered per dam in the high dose group was statistically significantly lower versus controls (9.9 vs 11.9) since the number of implants was lower. However, this number fits within the HCD (9.2 to 13.4) and no clear dose-effect relationship can be seen.

The live-birth indexes were between 99.3 and 95.9%: no dose dependent pattern was observed and the indexes were within the HCD for all doses. The lower index (95.9%) was observed in the higher dose of 200 mg/kg bw/d due to one dam with six out of nine still-born pups. The DS noted that the dam was "obviously unwell".

Offspring toxicity

A summary of data regarding the offspring toxicity in terms of survival ratio is given by the DS in the following table:

Parental generation	**F_0**				**F_1**			
Dose level [mg/kg bw/day]	**0**	**25**	**75**	**200**	**0**	**25**	**75**	**200**
Number of litters	25	25	24	25	25	25	25	22
- with live-born pups	25	25	24	25	25	25	25	21
- with still-born pups	1	1		1				1
Pups live-born	296	266	284	274	295	263	283	208
Pups found dead (day 1-4)	1	2	0	10	0	0	1	2
Pups cannibalized (day 1-4)	2	0	0	8	1	1	2	13
Pups PND 4 (pre-cull)	293	264	284	256	294	262	280	193
- Viability index [%]	99.1	99.4	100	93.5	99.7	99.6	99.0	93.6
Pups culled day 4	99	73	92	69	95	66	81	38
Pups PND 4 (post-cull)	194	191	192	187	199	196	199	155

Pups found dead (day 5-21)	0	0	0	0	0	0	0	0
Pups cannibalized (day 5-21)	0	0	1	0	0	1	0	1
Pups PND 21	194	191	191	187	199	195	199	154
- Lactation index [%]	100	100	99.0	100	100	99.5	100	99.4
Sex ratio [% live males], PND 0	47.7	49.5	51.3	49.2	49.4	44.4	52.8	50.2
Sex ratio [% live males], PND 21	47.6	50.7	47.8	50.2	48.9	48.0	50.8	48.7

Statistical analysis, viability and lactation indices: Wilcoxon with Bonferoni-Holm (1-sided), sex ratio: Wilcoxon test (2-sided); * $p \leq 0.05$, ** $p \leq 0.01$

Some toxicity indicators are comparable among all doses: the lactation index indicated that pup survival between PND 4 and 21 was high in all the groups and there was no evidence of a substance-related effect on the sex ratio. Other indicators were also comparable between the low and the middle dose groups but slight differences occurred at the high dose of 200 mg/kg bw/day as follows:

a) F_1 pups at 200 mg/kg bw/day
    1. the viability index had a value of 93.5 % (18 dead pups vs. 3 in the controls) as a consequence of one litter in which 11 pups died or were subject to cannibalism; the only pup that could be examined had indications of improper nursing (empty stomach). The remaining seven dead pups (two found dead, five cannibalised) originated from the litters of six additional F_0 dams. However, the viability index fits within the given HCD range: 89.4 % – 100 %;
    2. no substance-related clinical observations were apparent in the F_1 pups;
    3. the body weights were lower on PND 1 (about 9 % below controls) and remained lower until weaning (about 10 % below controls on PND 21). Accordingly, mean body-weight change was below the controls by up to 15 % throughout lactation;

b) F_2 pups at 200 mg/kg bw/day
    1. the viability index was 93.6% as a consequence of a higher number of pups dying during PND 1–4 (15 vs. 1 in the controls). Eleven of the 15 dead pups came from one litter and were not properly nourished (absence of milk in the stomach). The food consumption of this dam during PND 1–4 (13.3 g) was lower than the group mean of 33.3 g. The remaining four dead pups, which were subject to cannibalism, originated from 4 different litters of F_1 dams.
    2. no substance-related clinical observations were apparent in the F_1 pups with one exception: six pups from one dam showed evidence of reduced nutritional condition and absence of milk in the stomach on PND 1 and 2;
    3. the body weights were lower on PND 1 (about 7 % below controls) and remained lower until weaning (about 14 % below controls on PND 21). Overall, the mean body weight change was up to 19% lower than the concurrent controls throughout lactation.

The lower body weights were further reflected in the reduced organ weights following necropsy. The DS noted that there were slight differences between the absolute and relative values of brain, spleen and thymus; these were attributed to general decrease of the body weight.

The necropsy revealed the presence of the dilated pelvis in 23 F_2 pups (6 males, 17 females from 9 litters) at the higher dose. The finding appeared to be treatment-related since in the concurrent controls there were only three female pups from three litters. However, the presence of the dilated pelvis is not uncommon among the groups as shown in the following table:

	Male pups				**Female pups**			
Dose [mg/kg bw/day]	**0**	**25**	**75**	**200**	**0**	**25**	**75**	**200**
F_1 pups								
Animals examined	119	107	121	109	127	107	110	117
Renal pelvis, dilated	3 (3)	2 (2)	5 (4)	2 (2)	1	3 (3)	3 (3)	3 (3)
F_2 pups								
Animals examined	145	120	148	112	152	149	132	105
Renal pelvis, dilated		1	1	6 (5)	3 (3)	2 (2)	1	17 (9)

( ) values in brackets give litter incidence

The DS assumed that the finding was probably secondary to the general delay in development of the high-dose pups (up to 19% decrease in body weight gain); the study authors assumed this to be a largely reversible effect. It is noted that dilated renal pelvis is a visceral variation commonly encountered in rodent studies, predominantly in rats.

The anogenital distance was examined in PND 1 and in F_2 pups values slightly lower than in the controls were found in all groups; however, the index remained unchanged and very close to the controls.

The sexual maturation was determined in each male and female in the F_1 generation. The DS noted that the mean age at which females reached sexual maturity was slightly delayed in the high-dose pups (31.8 days compared with 30.0 days in controls), although there wasn't a clear dose-response relationship. The delay was also statistically significant in the low-dose (30.9 days) but not the mid-dose group (30.2 days). The days to vaginal opening was within the HCD in all the groups (15 studies, range 29.5 to 31.9). The age of preputial separation in male pups was not affected even in the high-dose group. Also, the examination of the males for nipples and areolae did not reveal any treatment-related effects.

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.

Supplemental information - In depth analyses by RAC

Study on rats

Mefentrifluconazole of 97.7% purity (conditioned in 1% carboxymethyl-cellulose as vehicle) was administrated by gavage to groups of 25 females corresponding to doses of 0, 50, 150 and 400 mg/kg bw/day during GD 6 to 19.

At terminal sacrifice day (GD 20), one female of each group had no implantation sites and were excluded i.e. 24 out of 25 females per group were pregnant and considered for the analysis.

Maternal toxicity was assessed and characterised by no deaths or clinical signs of toxicity in any group. The mean food consumption in the low and mid-dose remained unchanged while in the high dose group was reduced by 8% during GD 6-19. Consequently, the mean body-weight gain was about 17% below the controls. At sacrifice, the mean weights of the unopened uteri were comparable between groups. However, the body weight change corrected for gravid uterine weight was statistically significantly lower at 400 mg/kg bw/day (about 34 % below the concurrent control value), as was the carcass weight of the dams of this group (about 7% below controls). These changes were not recorded at lower doses.

There were no dose-related differences between control and test groups in the mean number of pre- and post-implantation losses, and in the number of resorptions or viable foetuses. All values were within the range of the HCD. The mean placenta weights were slightly but statistically significantly increased at 400 mg/kg bw/day (0.5 ± 007 g corresponding to approximately 113% of the control group value). However, the value was within the HCD and was not associated with impaired foetal development. Also, there were no changes in the sex ratio.

When referring to the developmental endpoints for litters with live foetuses, the mean foetal weights were not affected except for a decrease only in female pups at 400 mg/kg bw/day (3.4 vs. 3.6 g, 94% of control value). However, this value was within HCD. The malformations and variations were recorded as described below.

External malformations were recorded in one foetus at the 150 mg/kg bw/day (mandibular micrognathia with severely malformed skull bones) and one foetus at the 400 mg/kg bw/day dose (cleft palate, microphthalmia, malformed mandible). These findings were within the provided HCD range of 0-2%. With respect to the external variations, one case of limb hyperflexion was recorded at the low dose of 50 mg/kg bw/day.

Soft tissue variations were noted, one in control and another in each of the exposed groups; the findings were not statistically significant.

Dilated renal pelvis was noted with a litter incidence of 2 (8.3 %), 1 (4.2 %), 1 (13 %) and 5 (21 %) in the controls and dose groups respectively. In the provided HCD the range for the litter incidence was 0-57.1 % (mean 11.9 %); therefore, although apparently notable, the values were within the range of the HCD.

Skeletal variations, as given by the DS, are grouped in the following table:

Dose (mg/kg bw/day)		**0**	**50**	**150**	**400**
Foetuses evaluated		117	115	120	117
Live		117	115	120	117
Supra-occipital hole(s)					
Foetal incidence	# (%)	8 (6.8)	6 (5.2)	21 (18)	17 (15)
Litter incidence	# (%)	6 (25)	5 (21)	**15 (63)****	**13 (54)***
Affected foetuses / litter	%	6.5 ± 12.18	5.1 ± 10.81	**18.4 ± 19.24****	**17.9 ± 23.44***
Misshapen sacral vertebra					
Foetal incidence	# (%)	3 (2.6)	5 (4.3)	7 (5.8)	12 (10)
Litter incidence	# (%)	3 (15)	3 (13)	5 (21)	**9 (38)***
Affected foetuses / litter	%	2.7 ± 7.37	3.9 ± 10.79	5.8 ± 12.55	**10.9 ± 18.06***
Dumb-bell ossification of thoracic centrum with dumb-bell-shaped cartilage of centrum					
Foetal incidence	# (%)	7 (6.0)	8 (7.0)	9 (7.5)	18 (15)
Litter incidence	# (%)	6 (25)	7 (29)	7 (29)	10 (42)
Affected foetuses / litter	%	5.6 ± 10.83	7.1 ± 11.90	7.8 ± 14.82	14.2 ± 20.63

Statistics, litter incidence: Fisher's Exact Test (1-sided); affected foetuses/litter: Wilcoxon-Test (1-sided)
* : $p \leq 0.05$; ** : $p \leq 0.01$

The statistically significant values are presented in bold text. Depending on how the findings are reported, some values might suggest a dose-related relationship, such as for the foetal incidence and foetuses/litter for the misshapen sacral vertebra. However, overall there is no clear relationship; moreover, all the foetal incidences, litter incidences and mean affected foetuses / litter were within the associated HCD given in the following table:

	Foetuses (7684)			**Litters (1431)**			**Affected foetuses / litter**	
	No.	%	Range	No.	%	Range	% Mean	Range
Supra-occipital holes	954	12.4	0.0 – 52.3	521	36.4	0.0 – 100.0	12.2	0.0 – 50.8
Misshapen sacral vertebra	250	3.3	0.0 – 10.2	212	14.8	0.0 – 41.7	3.4	0.0 – 10.9
Dumb-bell ossification of thoracic centrum with dumb-bell-shaped cartilage of centrum	318	4.1	0.0 – 16.2	249	17.4	0.0 – 56.0	4.3	0.0 – 19.4

76 studies performed at the test facility (2009–2014) with Wistar rats (Charles River) (1431 litters with 7684 viable foetuses examined)

With respect to the nomenclature used in the evaluation, the DS considered that the 'misshapen sacral vertebra' represent a slight developmental delay without any adverse effects on development and thus do not provide evidence of developmental toxicity.

When all the external, visceral and skeletal results are grouped the following total incidences were observed:

Dose (mg/kg bw/day)		**0**	**50**	**150**	**400**
Litters evaluated		24	24	24	24
Foetuses evaluated		223	221	230	222
Live		223	221	230	222
Total malformations					
Foetal incidence	# (%)	3 (1.3)	0 (0.0)	1 (0.4)	2 (0.9)
Litter incidence	# (%)	3 (13)	0 (0.0)	1 (4.2)	2 (8.3)
Affected foetuses / litter	%	1.3 ± 3.41	0.0 ± 0.00	0.5 ± 2.27	1.0 ± 3.68
Total variations					
Foetal incidence	# (%)	119 (53)	116 (52)	123 (53)	124 (56)
Litter incidence	# (%)	24 (100)	24 (100)	24 (100)	24 (100)
Affected foetuses / litter	%	53.5 ± 4.49	52.7 ± 3.52	53.5 ± 3.66	55.7 ± 8.13

Statistics, litter incidence: Fisher's Exact Test (1-sided); affected foetuses/litter: Wilcoxon-Test (1-sided)
* $p \leq 0.05$, ** $p \leq 0.01$

Overall, there were no statistically significant findings.

Study on rabbits

Dose range-finding studies

Three dose range-finding (DRF) studies were performed in preparation of the prenatal developmental toxicity study on rabbits. These studies are not formally rated as GLP compliant but it is noted that all the procedures (*e.g.* observation periodicity, food consumption etc.*)*

were GLP compliant and the test facility was accredited accordingly. All the studies were performed on New Zealand White rabbits by gavage administration. The following specific details were provided by the DS:

A. DRF #1. Groups of three non-pregnant females rabbits were administered mefentrifluconazole (prepared in 1% aqueous carboxymethyl-cellulose as vehicle) at dose levels of 0, 50, 150, or 400 mg/kg bw/day, once daily for a duration of 21 days (comparable to the 23 treatments of a OECD TG 414 study with pregnant rabbits during GD 6-28). The top dose level was chosen based on maternal toxicity findings in rats.
    a. At the low dose of 50 mg/kg bw/day two out of three does were sacrificed in a moribund condition on days 14 and 20, and one survived until day 21. All animals showed poor or reduced nutritional condition. Measured food intake was between 20.9–85.4 g on day 1, reduced to 0–12.7 on day 2 and < 4.3 g on the remaining days in all rabbits. After the fourth treatment, the animals showed reduced or no faeces. At necropsy, the small intestines were found empty in all rabbits, while the large intestines were found empty and no faeces were present in rectum in two out of the three rabbits. Erosion of glandular or forestomach was recorded in two rabbits and a small spleen in the third.
    b. The 150 mg/kg bw/day dose was reduced after three treatments to 15 mg/kg bw/day starting on day 3 due to low food intake. However, all three rabbits were sacrificed moribund on day 4, after being dosed with 3x 150 and 2x 15 mg/kg bw/day. At necropsy, the small intestines were found empty, the large intestines were watery, and no faeces were found in the rectum of all rabbits. In addition, two rabbits had an enlarged urinary bladder.
    c. At the top dose of 400 mg/kg bw/day, one rabbit was found dead on day 2 and two rabbits were killed moribund on day 2. Compared to the normal food consumption of 150 ± 50 g/day in control group rabbits, the first animal showed almost no food intake (0.7 g, day 0-1) and, at necropsy multiple erosions were observed in its forestomach. The other two animals showed watery contents in the large intestine, and one of the rabbits had no faeces in the rectum, demonstrating a drastic decrease of food intake.
B. DRF #2. A single group of three rabbits were administrated a single dose of 25 mg/kg bw/day in conditions similar to DRF #1. Two rabbits showed no treatment-related findings (no clinical signs, normal food intake and body development) and no findings were observed at gross necropsy. The third was sacrificed in moribund condition on day 17. The animal had reduced food intake; at necropsy, the small and large intestines were empty, the stomach contained dry, hard food and the rectum contained no faeces.
C. DRF #3. Five artificially inseminated rabbits were administered mefentrifluconazole from GD 6 through GD 28. The dose levels were 0, 5, 10 and 20 mg/kg bw/day. The resulting pregnancy rates were 3/5, 4/5, 5/5 and 4/5 at 0, 5, 10 and 20 mg/kg bw/day, respectively. No clear treatment-related adverse effects were noted in the study up to 20 mg/kg bw/day, the highest dose tested.

The DS concluded on the three DRFs that the effects observed at mefentrifluconazole dose levels that resulted in drastically reduced food intake are considered to be related to rabbit-specific gastrointestinal physiology and digestion features, which are not relevant to humans.

The developmental study

The same OECD TG 414 compliant protocol was used in a study on rabbits. Mefentrifluconazole of 97.7% purity (conditioned in 1% aqueous carboxymethyl-cellulose as vehicle) was administrated by gavage to groups of 30-33 females corresponding to doses of 0, 5, 15 and 25 mg/kg bw/day during the GD 6 to 28. The doses were established based on three dose-range finding studies presented above.

At sacrifice on GD 29, only 20 to 24 does per group had implantation sites but this is in accordance with a minimum of 16 females/group specified in test guideline. The non-pregnant does were excluded from the calculations.

Maternal toxicity was not evident in all groups. The deaths registered in both controls and high-dose groups were attributed exclusively to gavage errors. Two spontaneous abortions were noted in one low-dose and one mid-dose female, respectively. Also, there were no clinical signs of toxicity. Overall food consumption values were comparable in all groups for the entire duration (days 0-29): 100%, 106% and 96% of control values at 5, 15 and 25 mg/kg bw/day. Also, the mean body weights and body weight changes were not statistically significant: 91 %, 100 % and 87 % at 5, 15 and 25 mg/kg bw/day, respectively. Clinical chemistry revealed decreased activity of ALT and AST in the high-dose group (ALT by 24%, AST by 34%) and of AST only in the mid-dose group. Since no other liver parameters were changed, the DS concluded they were treatment-related but not adverse. Also, the decreased level of globulin in the high-dose was seen as an adaptive rather than adverse effect. No other changed clinical-chemistry parameters were noted.

In litters with implants, there were no treatment-related effects on the number of pre- and post-implantation losses or number of resorptions. However, at 15 mg/kg bw/day, the number of implantation sites (mean of 7.0 per dam) and subsequently the live-litter size (mean of 6.4 per dam) was statistically significantly lower than the controls and slightly below the HCD. Since these values resulted from a mean number of *corpora lutea* below the HCD and a higher (but within HCD) pre-implantation loss, the finding was considered not to have been treatment related. Furthermore, there was no dose-response relationship in the number of implantation sites and live-litter size, and no adverse findings in the reproductive organs of the affected animals. All other differences observed reflected the normal range of fluctuations for animals of this strain and age; this included a higher post-implantation loss in the control and 15 mg/kg bw/day groups, which were caused by a single dam in each group with spontaneously-resorbed litters. The mean number and weight of live foetuses, of male and female foetuses, the sex ratio and placental weights were not affected by treatment.

In litters with live foetuses, the mean foetal weights were fully comparable among all groups. The malformations and variations were recorded as described below.

There were no treatment related visceral malformations, visceral variations and soft-tissue unclassified findings; none of the findings in this category were either consistent or related to the doses.

Skeletal malformations were recorded in the exposed as well as in the control groups. Skeletal variations of different bone structures were observed, with or without effects on the corresponding cartilage, in all groups, but without a dose-response relationship. None of these findings were statistically significant; also, there was only one case in which the finding was not within the HCD. One finding that was classified by the study authors as a skeletal variation (fused sternebra with unchanged cartilage) occurred with a higher incidence in the high-dose

group than the controls, although without statistical significance. The data regarding the fused sternebra are given by the DS in the following table:

Dose (mg/kg bw/day)		**0**	**5**	**15**	**25**
Litters evaluated		23	20	21	22
Foetuses evaluated		194	161	135	176
Live		194	161	135	176
Fused sternebra; unchanged cartilage					
Foetal incidence	#(%)	1 (0.5)	3 (1.9)	1 (0.7)	7 (4.0)
Litter incidence	#(%)	1 (4.3)	2 (10)	1 (4.8)	**4 (18)**
Affected foetuses / litter	%	0.5 ± 2.32	3.3 ± 10.26	0.5 ± 2.42	5.5 ± 15.4

Statistics, litter incidence: Fisher's Exact Test (1-sided); affected foetuses/litter: Wilcoxon-Test (1-sided)
* : $p \leq 0.05$; ** : $p \leq 0.01$

The DS noted that the fused sternebra (unchanged cartilage) at 18 % (4/22 litters) in the high dose group represented only one litter above the historical-control range (14.3 % for 3/21 litters in the HCD). Based on the lack of a dose-response relationship and statistical significance for the finding, the DS concluded that the slightly higher incidence of this variation in the high-dose group does not constitute evidence of a developmental effect. Furthermore, there were more cases of a malformed sternum in the controls than in the high-dose group, suggesting that both effects are probably not treatment related.

The overall data regarding the malformations and variations in the rabbit study is shown in the following table:

Dose (mg/kg bw/day)		**0**	**50**	**150**	**400**
Litters evaluated		23	20	21	22
Foetuses evaluated		194	161	135	176
Total malformations					
Foetal incidence	# (%)	8 (4.1)	3 (1.9)	2 (1.5)	3 (1.7)
Litter incidence	# (%)	6 (26)	3 (15)	2 (9.5)	3 (14)
Affected foetuses / litter	%	3.8	2.1	1.4	1.7
Total variations					
Foetal incidence	# (%)	191 (98)	160 (99)	134 (99)	173 (98)
Litter incidence	# (%)	23 (100)	20 (100)	21 (100)	22 (100)
Affected foetuses / litter	%	98.7	99.4	99.5	98.4

Statistics, litter incidence: Fisher's Exact Test (1-sided); affected foetuses/litter: Wilcoxon-Test (1-sided)
* $p \leq 0.05$, ** $p \leq 0.01$

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 they 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.

10.11 Specific target organ toxicity-single exposure

The available acute studies that inform on specific target-organ toxicity following a single exposure are reported in section 10.1 to 10.3. An acute neurotoxicity study in rats is also available (summarised in the table below).

Table 47. Summary of acute neurotoxicity study with mefentrifluconazole

Study **Batch / purity**	**Doses**	**Main adverse effects**
Acute neurotoxicity **Wistar rat** 10 / sex / group Oral gavage; vehicle: 1% CMC OECD 424; GLP Purity 98.6% [redacted] 2015	0, 200, 600, 2000 mg/kg bw Observation period: 14 days	2000 mg/kg bw ↓ body-weight gain (males & females) during days 0-7 ↑ unsteady gait of 5/10 males and 3/10 females on day 0 ↓ motor activity (males & females) on day 0 ↓ male forelimb grip strength on day 0 ↑ male landing foot splay on day 0

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

In an acute neurotoxicity study in which mefentrifluconazole was administered orally at doses up to 2000 mg/kg bw, there were no deaths or clinical signs of toxicity during the clinical examinations in any group. At 2000 mg/kg bw, mean body-weight gains were lower in males (-29%, statistically significant) and females (-20%, not statistically significant) between study days 0 to 7. Mean body weights of treatment groups were not significantly different from controls. The animals recovered between study days 8 to 14, such that by the end of the study period neither body-weight gain nor body weight was affected. There were no effects at 200 and 600 mg/kg bw.

Functional observation battery (FOB) measurements were taken prior to administration and on study days 0, 7 and 14. Home-cage observations were negative at all time-points for all groups. On study-day 0, slight impairment of coordination, i.e. unsteady gait, was observed at 2000 mg/kg bw in five male and three female animals, as compared to none in male and female control animals. These slight changes were related to the bolus dosing of the relatively high, limit-dose level. It did not occur on study days 7 and 14. No changes were observed on study-days 7 and 14, nor at dose levels of 200 or 600 mg/kg bw on any study day. There were no treatment-related effects in the sensorimotor tests and reflexes.

On the day of test-substance administration, treatment-related effects in the quantitative test parameters were observed in the high-dose male animals. In this group, grip strength of the forelimbs was lower than the controls (-22 %). In addition, the landing foot-splay test revealed a statistically significantly increased distance between the hind-limbs (12.5 cm compared with 9.3 cm in the controls). Both these findings indicated a lower body tension. Since there were no (histo-)pathological findings and the effect was not evident on days 7 and 14 after administration, the dossier submitter concludes that the change was related to general toxicity and impaired well-being on the day of treatment, not to structural neuronal damage. In mid-dose males, landing foot-splay distance was increased between the hind-limbs, but the mean value of 11.6 cm was within the historical control range (10 acute neurotoxicity studies conducted between 2010-2013, range of study means in male Wistar rats (Crl:WI(Han)) = 7.5 - 12.0 cm). The change was thus assessed to be incidental. No findings were observed for female animals at any dose.

Motor activity was measured on the same days as the FOB was performed. The only treatment-related changes occurred on the day of mefentrifluconazole administration. The mean values for overall motor activity were

statistically significantly reduced in male and female animals at 2000 mg/kg bw and in females at 600 mg/kg bw. Neuropathology evaluation was performed on five animals per sex per group. Terminal body weight and absolute and relative brain weights were unaffected at all doses. There were no treatment-related gross or histopathology findings.

In conclusion, in an acute oral neurotoxicity study, administration of mefentrifluconazole at a dose of 2000 mg/kg bw resulted in some neuro-behavioural effects on the day of dosing. All these effects were transient and unrelated to structural or functional neuronal damage. The dossier submitter therefore concludes that they were related to systemic toxicity and impaired well-being subsequent to the application of a high-dose bolus application of test substance.

In an acute oral study in rats (section 10.1), mefentrifluconazole was administered at a single dose level of 2000 mg/kg bw. The observed clinical signs (cowering position, impaired general state and piloerection) were indicative of general toxicity and did not give any indication of specific target-organ toxicity; moreover, they had resolved within a few hours of the dose being administered, and gross pathology did not reveal any adverse findings. In an acute dermal study in rats (section 10.2), a dose of 5000 mg/kg bw did not induce any clinical signs of systemic toxicity and there were no macroscopic findings at necropsy.

In an acute inhalation toxicity study in rats (section 10.3), animals were exposed (head and nose) to a dust aerosol of mefentrifluconazole at a concentration of 5.314 mg/L for 4 hours. Clinical signs of toxicity included laboured respiration, abdominal respiration, respiration sounds, encrusted eyes, red and colourless discharge and/or red crusts of the nose. These general indicators of toxicity and respiratory effects that are commonly associated with the inhalation route of exposure were observed between 2 hours and 11 days after the exposure. No clinical symptoms were recorded from day 12 onwards. The results of this study do not provide specific evidence of an irritant effect on the respiratory tract of rats.

There were no indications of narcosis or impaired consciousness in any of these studies.

10.11.2 Comparison with the CLP criteria

STOT-SE categories 1 and 2 are assigned on the basis of clear evidence of significant or severe toxicity to a specific target organ that arises from a single exposure to a substance. STOT-SE category 3 is currently assigned for the transient effects of respiratory tract irritation and narcotic effects.

The available acute studies do not provide any indication that mefentrifluconazole meets the classification criteria for specific target-organ toxicity category 1, 2 or 3 following a single exposure. No classification is proposed.

10.11.3 Conclusion on classification and labelling for STOT SE

Not classified (conclusive but not sufficient for classification).

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

Summary of the Dossier Submitter's proposal

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).

10.12 Specific target organ toxicity-repeated exposure

The specific target-organ toxicity of mefentrifluconazole upon repeated exposure has been investigated in 28-day and 90-day studies in rats, mice and dogs and a one-year study in dogs. Additional information is provided by chronic / carcinogenicity studies in rats and mice, which are reported in section 10.9.

Table 48: Summary table of animal studies on repeated-dose toxicity of mefentrifluconazole

Method Guideline, Deviation(s) from the guideline (if any)	**Species Strain Sex no/group**	**Dose levels duration of exposure**	**CLP guideline value for classification (mg/kg bw/d, rat study)**	**Results**
28-day oral (dietary) OECD 407 (2008) GLP Purity 97.7% [redacted] 2015a	Rat, Wistar 5/sex/group	0, 500, 1500, 4000 ppm Equivalent to Males: 0, 47, 135, 388 mg/kg/d Females: 0, 47, 138, 334 mg/kg/d	Cat 1 = 30 Cat 2 = 300	No treatment-related deaths or overt signs of toxicity in any dose group <u>47 mg/kg bw/d</u>: No adverse effects <u>135/138 mg/kg bw/d</u>: No adverse effects <u>388/334 mg/kg bw/d:</u> ↓ body-weight gain (by ~ 30 %** in males & females) and food intake (females); final body weight ↓ by 15 % & 9 % in males & females, respectively ↓ albumin (females, by 7 %**), ↓ total bilirubin (females, by 66 %*), ↑ cholesterol (females, by 75 %**) ↑ relative liver weight in females (by 23 %**), ↓ absolute kidney weight in males (by 12 %*) ↑ liver cell hypertrophy (minimal severity – grade 1): 2/5 males, 5/5 females compared with 0/5 in each control group
28-day oral (dietary) OECD 407 (2008) GLP Purity 95.5% [redacted] 2014a	Mouse, C57BL/6 Rj 5/sex/group	0, 30, 100, 300, 1000 ppm Equivalent to Males: 0, 4.8, 15.5, 47.9, 128 mg/kg/d Females: 0, 5.8, 18.5, 61.0, 145 mg/kg/d	Cat 1 = 30 Cat 2 = 300	No treatment-related deaths or overt signs of toxicity in any dose group <u>4.8 / 5.8 mg/kg bw/d:</u> ↑ relative liver weight (by 12 %* but within historical-control range) & liver cell hypertrophy in males (5/5, minimal) <u>15.5 / 18.5 mg/kg bw/d:</u> ↑ relative liver weight (by 18 %*), liver cell hypertrophy in males (5/5, 1 minimal, 4 moderate) <u>47.9 / 61 mg/kg bw/d:</u> ↑ relative liver weight (by 22 %* in males & 33 %* in females) & liver cell hypertrophy (5/5 for males minimal to moderate & 5/5 females minimal to slight, compared with 0/5 in each control group)

Method Guideline, Deviation(s) from the guideline (if any)	**Species Strain Sex no/group**	**Dose levels duration of exposure**	**CLP guideline value for classification (mg/kg bw/d, rat study)**	**Results**
				128 / 145 mg/kg bw/d: ↓ body-weight gain (overall by 65 % in females; weight loss in males) & food intake (males & females), ↓ final body weights (by 13 % males, 6 % in females), ↑ ovary weight (absolute & relative, 63-70 %) ↑ alanine aminotransferase (ALT) (males*), ↓ cholesterol (males**), ↓ glucose (females**). ↓ albumin (females*); marked ↑ liver weight (relative > 70 %**) Liver histopathology: hypertrophy = 5/5 males (centrilobular, moderate), 5/5 females (diffuse, slight) liver cell necrosis = 4/5 males (3 minimal, 1 slight), 3/5 females (2 minimal, 1 slight) oval cell proliferation = 2/5 males (minimal), 5/5 females (minimal) bile duct hyperplasia = 1/5 males (minimal), 5/5 females (minimal)
28-day oral (capsule) OECD 407 GLP Purity 98.6% [redacted] 2015a	Beagle dog (range-finding study) 3/sex/group	Males: Days 1-2: 300 or 1000 mg/kg bw/d Days 7-35/36: 125 or 250 mg/kg bw/d Females: Day 1: 300 or 500 mg/kg bw/d Days 3-29/30: 125 or 250 mg/kg bw/d	Cat 1 = 30 Cat 2 = 300	There were no deaths in any dose group 300 and 1000 mg/kg bw/d: Severe clinical signs in all dogs (males and females), comprising vomitus, impaired general condition, unsteady gait, reduced food intake within one to two days of first dose. Consequently, doses were reduced. 250 (reduced from 1000 /500) mg/kg bw/d 2-3 dogs/sex with delayed food intake; isolated vomiting, single occurrence of unsteady gait and poor general condition ↓ body-weight gain (-0.5** / -0.9* kg in males/females on days 0 – 14; overall -0.3* / -0.7** kg males/females), ↓ cholesterol (by 45 %) 1 female with ↑ aspartate aminotransferase (AST) & ALT, ↓ terminal body weight (-12%) ↑ liver weight (relative ≥ 31 %) with hypertrophy and eosinophilic change of hepatocytes 125 (reduced from 300) mg/kg bw/d 1 female with delayed food intake 1 male with vomiting on 3 days

Method Guideline, Deviation(s) from the guideline (if any)	**Species Strain Sex no/group**	**Dose levels duration of exposure**	**CLP guideline value for classification (mg/kg bw/d, rat study)**	**Results**
				↓ body-weight gain (-0.3* kg males, days 0 - 14), ↓ cholesterol (by 43 %) ↓ terminal body weight (-5 to -7%) ↑ liver weight (relative ≥ 25%) in males & females with hypertrophy and eosinophilic change of hepatocytes
90-day oral (dietary) OECD 408 GLP Purity 95.5% [redacted] 2015b	Rat, Wistar 10/sex/group	0, 400, 1200, 3600 ppm Equivalent to: Males: 0, 27, 76, 256 mg/kg bw/d Females: 0, 30, 91, 314 mg/kg bw/d	Cat 1 = 10 Cat 2 = 100	There were no deaths or overt clinical signs of toxicity <u>27 / 30 mg/kg bw/d</u> No adverse effects <u>76 / 91 mg/kg bw/d:</u> No adverse effects <u>256 / 314 mg/kg bw/d:</u> ↓ body-weight gain (males: -11%, females: -20%**) ↑ alkaline phosphatase (ALP) (males** & females**), ↑ cholesterol* + ↓ albumin** (females) ↑ relative liver weight (males: +11%**, females: +13%**) ↑ minimal hepatocellular hypertrophy (8/10 males & 3/10 females, minimal)
90-day oral (dietary) OECD 408 GLP Purity 98.8% [redacted], 2015a [redacted] 2014a (plasma analysis) [redacted] 2015a (amendment to plasma analysis to correct typing error)	C57BL/6 Rj mouse 15/sex/dose	0, 10, 50, 250, 750 ppm Males: 0, 2, 11, 58, 174 mg/kg bw/d Females: 0, 3, 15, 67, 211 mg/kg bw/d	Cat 1 = 10 Cat 2 = 100	There were no deaths or overt clinical signs of toxicity <u>2 / 3 mg/kg bw/d:</u> No adverse effects <u>11 / 15 mg/kg bw/d:</u> ↑ haemoglobin & haematocrit in males** ↓ cholesterol in males** <u>58 / 67 mg/kg bw/d:</u> In males, ↑ haemoglobin, haematocrit, mean corpuscular haemoglobin (MCH), red blood cell & platelet counts (evidence of haemoconcentration) ↓ cholesterol in males** & females**, ↓ albumin/globulin ratio in females* ↑ relative liver weight (males: +38%**, females: +26%**) & hypertrophy ↑ liver single-cell necrosis (grade 1) in 2/10 males & cytoplasmic alteration (grade 1) in 4/10 males <u>174 / 211 mg/kg bw/d:</u> ↓ body-weight gain (consistent in males**, transient in females) ↑ platelet, ↓ relative eosinophil counts in females ↓ albumin/globulin ratio in males* & females**

Method Guideline, Deviation(s) from the guideline (if any)	**Species Strain Sex no/group**	**Dose levels duration of exposure**	**CLP guideline value for classification (mg/kg bw/d, rat study)**	**Results**
				↑ relative liver weight (males: +87%**, females: +67%**) Liver histopathology: liver cell necrosis = 8/10 males, single cell, 7 minimal, 1 slight; 6/10 females, (multi)focal, 2 minimal, 4 slight cytoplasmic alteration = 5/10 males, 3 minimal, 2 slight; 2/10 females, 1 minimal, 1 slight
90-day oral capsule OECD 409 (1998) GLP Purity 98.6% [redacted] 2015a	Beagle dog 5/sex/dose	0, 15, 90, 180 mg/kg bw/d	Cat 1 = 10 Cat 2 = 100	There were no deaths <u>15 mg/kg bw/d:</u> No adverse effects <u>90 mg/kg bw/d:</u> No adverse effects <u>180 mg/kg bw/d</u>: 1 male and 3 females with vomiting & delayed food intake ↓ food intake (females) (max. -7%, day 7) ↓ body-weight gain (days 0-91 males: -49.4%*, females: -59.6%) ↑ ALP (3 months, males* & females*) ↓ protein (males: 6 weeks**, females: 6 weeks* & 3 months*); ↓ creatinine (females, 6 weeks) ↑ relative liver weight (males: +20%**)
12-month oral capsule OECD 452 GLP Purity 98.8% [redacted] 2016	Beagle dog 5 / sex / dose	0, 10, 30, 150 mg/kg bw/d	Cat 1 = 2.5 Cat 2 = 25	<u>10 mg/kd bw/d:</u> Liver: minimal eosinophilic change (3/5 males, 2/3 females) <u>30 mg/kd bw/d:</u> ↑ liver weight in males (relative +11 %) and females (relative + 18 %) Liver: centrilobular hepatocellular hypertrophy, minimal or slight (3/5 males, 2/5 females); minimal eosinophilic change (3/5 males, 3/5 females) <u>150 mg/kg bw/d:</u> ↓ body weight in females (up to -11.6 %) ↑ ALP in males & females; ↓ AST in males ↓ total protein (males), albumin (males & females), calcium (males & females), creatinine (females) ↓ absolute lymphocyte counts (males at 3 months)

Method Guideline, Deviation(s) from the guideline (if any)	**Species Strain Sex no/group**	**Dose levels duration of exposure**	**CLP guideline value for classification (mg/kg bw/d, rat study)**	**Results**
				↑ absolute & relative liver weight in males (relative +33 % *) and females (relative +31 %) Liver: centrilobular or diffuse hepatocellular hypertrophy in 5/5 males & 5/5 females; minimal eosinophilic change (5/5 males, 5/5 females)
28-day dermal OECD 410 GLP Purity 98.6% [redacted] 2015b	Wistar rat 10/sex/dose	0, 100, 300, 1000 mg/kg bw/d 6 hours/day on 5 days / week for 4 weeks (males: 21 applications; females: 22 applications) in 0.5 % carboxy-methyl-cellulose in drinking water	Cat 1 = 20 Cat 2 = 200	No adverse systemic or local effects at any dose

* = statistically significant, $p \leq 0.05$; ** = statistically significant, $p \leq 0.01$

Oral

Rats

Dietary administration of mefentrifluconazole to Wistar rats for 28 days resulted in reduced body-weight gains in males and females at 388 / 334 mg/kg bw/d, resulting in lower body weights at the end of the study; reduced feed consumption was additionally recorded in females. Indications of slight impairment of liver function were reported in females, with increased relative weight, centrilobular hepatocellular hypertrophy of minimal severity and slight alterations in clinical pathology parameters (albumin and cholesterol) at 388 / 334 mg/kg bw/d; however, no adverse findings were noted in the liver upon histopathology.

Oral administration to Wistar rats for 90 days resulted in statistically significantly reduced body-weight gain and final body weight in females of the high-dose group (314 mg/kg bw/d). Also at this dose, increased relative liver weights in males and females, minimal hepatocellular hypertrophy and changes in clinical-chemistry parameters indicated that there was a slight dysregulation of liver-cell function. There were no adverse findings in the mid- and low-dose groups.

Mice

Dietary administration of mefentrifluconazole to mice for 28 days resulted in reduced body weights and body-weight gain, clinical chemistry changes, liver toxicity and increased ovary weight at 128 / 145 mg/kg bw/d. The liver was identified as a target organ. Besides the histopathology (hepatocellular necrosis) at this dose, there were statistically-significant changes in relative liver weights in males at all doses, although the value at 4.8 / 5.8 mg/kg bw/d was within the historical-control range. In females, statistically significant increases in relative liver weights occurred from 61 mg/kg bw/d. Apart from hepatocellular hypertrophy, which is a morphological description and not in itself an indication of adversity, neither adverse liver histopathology

findings nor clinical chemistry changes were observed at doses lower than 128 / 145 mg/kg bw/d, and so it could be argued that the liver-weight increases at these doses were not adverse.

When administered to mice at doses of 174 / 211 mg/kg bw/d for 90 days, mefentrifluconazole caused reductions in body weight gain and clear signs of hepatotoxicity in both sexes, including changes in several clinical chemistry parameters, pronounced liver weight increases with associated hepatocellular hypertrophy, liver cell necrosis and degenerative hepatocellular (cytoplasmic) changes. In the next dose group (58 mg/kg bw/d in males and 67 mg/kg bw/d in females), degenerative liver changes of minimal severity were observed in 6 of 10 male mice; liver-weight increases, hepatocellular hypertrophy and reduced cholesterol were recorded in both sexes. The toxicological relevance of increased levels of red blood cell parameters and platelets in male mice at 58 mg/kg bw/d was not clear and may have been caused by decreased water consumption or stress. At 11 / 15 mg/kg bw/d, very slight increases in haemoglobin and haematocrit and a reduction in cholesterol were observed in male mice and were considered to be treatment-related. However, since the haematology changes at this dose were ≤ 5 % and did not occur in females, the dossier submitter does not consider them convincing evidence of an adverse effect. Additional treatment-related findings in male mice at this dose comprised slightly increased liver weights with associated liver-cell hypertrophy of minimal severity in 8 of 10 males. The dossier submitter considers these liver changes to be an adaptive response to mefentrifluconazole exposure and not adverse. In females at 15 mg/kg bw/d and both sexes at 2 / 3 mg/kg bw/d, treatment-related effects were confined to slightly reduced cholesterol levels only, which in isolation are not regarded as adverse by the dossier submitter.

Dogs

The 28-day dog study was intended to be a range-finding study and thus employed only three animals per sex per group and only two doses. The initial doses administered resulted in severe clinical signs (vomitus, impaired general condition, unsteady gait, reduced food intake) within one to two days which necessitated a reduction in the dose levels to 125 and 250 mg/kg bw/d. Adverse effects were seen at both dose levels and comprised clinical signs, decreased body weight and body-weight gain and increased relative liver weight with histopathology changes (hypertrophy and eosinophilic change of hepatocytes, both of minimal-to-slight severity).

The oral administration of mefentrifluconazole by capsule to male and female Beagle dogs for 90 days caused test substance-related, adverse signs of toxicity at a dose level of 180 mg/kg bw/d that comprised reductions in food intake, body weight and body-weight gain, increased alkaline phosphatase, decreased serum protein concentration in both sexes, transiently decreased creatinine in females and increased liver weight in males. At the mid-dose level of 90 mg/kg bw/d, the only treatment-related clinical pathology change was a slightly increased alkaline phosphatase activity in males, but since at this dose the increased liver weight did not show a dose-response relationship and there were no other indications of hepatotoxicity, the dossier submitter does not consider this to be an adverse effect. Thus, the only clear target organ was the liver. Although changes in the weights of the testes and prostate were noted in animals exposed to mefentrifluconazole, these were for the main part without statistical significance, which reflected the shallow dose-response curves and substantial variability in values within all the groups. Furthermore, there were no pathology findings to explain the testes weight changes. The prostate was reduced in size in 1 / 5 males at 90 mg/kg bw/d and 2 /5 males at 180 mg/kg bw/d. However, this reduction in size did not correlate with any histopathology findings. Overall, the dossier submitter concludes that these organ-weight changes do not provide robust evidence of a treatment-related adverse effect.

In the one-year dog study, there were no overt clinical signs of toxicity at doses up to the maximum tested of 150 mg/kg bw/d. The main target organ was the liver, with treatment-related increases in the relative weight at 30 and 150 mg/kg bw/d. At both doses, these weight increases were accompanied by histopathological changes that comprised hepatocellular hypertrophy; the hypertrophy had a centrilobular or diffuse distribution pattern in the high-dose animals but only a centrilobular pattern in the mid-dose animals. The adverse nature of the effects at 150 mg/kg bw/d was suggested by the clinical chemistry findings, in which treatment-related increased ALP activities in both sexes and decreased AST activities in males at 150 mg/kg bw/d were recorded. In the absence of such changes at 30 mg/kg bw/d, the increases in liver weight that were at the margin of the threshold for adversity and accompanied by minimal to slight hepatocellular hypertrophy were likely to have

been adaptive. In the high-dose animals, the hypertrophic hepatocytes showed a pale eosinophilic, finely granular cytoplasm with less-pronounced vacuolation than that of the controls. Some of the animals of the low- and mid-dose groups had an eosinophilic change as described above, but without a change in the cell size. The livers of all males were stained with periodic-acid-Schiff (PAS) to detect glycogen; no difference was detected in the amount of glycogen between the controls and the exposed groups. The less-pronounced vacuolation was thus interpreted by the study authors to be part of the eosinophilic change and the hepatocellular hypertrophy rather than a separate pathological finding. Besides changes in the liver weight, at 150 mg/kg bw/d statistically significant increases in absolute and relative adrenal-gland weights in females and relative kidney weights in both sexes were recorded. The weight changes in the adrenal glands were not accompanied by any gross or histopathology changes. Histopathology of the kidneys revealed a slightly decreased number of cytoplasmic vacuoles in the cortical tubular epithelial cells of the inner cortex of high-dose females than the other groups. An oil-red-O stain on the kidneys of single animals of each group demonstrated that the vacuoles were lipid droplets. The decreased lipid storage probably occurred as a consequence of lower body-weight gains in this group. There were no statistically significant, dose-related changes in the weights of the ovaries, uterus, prostate, epididymides, pituitary gland or testes, nor gross or histopathology findings in these organs.

Dermal

Dermal administration of mefentrifluconazole up to the limit dose of 1000 mg/kg bw/d for 28 days did not result in any adverse local or systemic effects in rats.

Additional information on repeated-dose toxicity

Studies to investigate the chronic toxicity and carcinogenicity potential of mefentrifluconazole have been conducted in rats and mice (section 10.9).

In a combined chronic / carcinogenicity rat study, there were no deaths in any of the satellite groups in the chronic phase of the study (12 months' exposure). In the main, carcinogenicity, groups (two years' exposure), there was not a treatment-related increase in deaths. There were no overt clinical signs of toxicity in any group in either the satellite or main cohorts. Body weights and body-weight gain were reduced in the high-dose male and female groups after both 12 (191 / 300 mg/kg bw/d in males and females, respectively) and 24 months (163 / 302 mg/kg bw/d in males / females) of exposure from day 7 and then throughout the duration of the study.

Treatment-related changes in haematology parameters consisted of slight decreases in the activated partial thromboplastin time (PTT) in males at 31 mg/kg bw/d and both sexes at 191 / 300 mg/kg bw/d, and a decrease in platelet counts in males at 191 mg/kg bw/d (12 months). Several treatment-related changes in clinical-chemistry parameters were recorded at 191 / 300 mg/kg bw/d, which indicated that adaptive changes and some alterations to liver-cell metabolism resulted from exposure to mefentrifluconazole. The liver weight relative to body weight was statistically significantly increased in high-dose males (by 9 %) and females (by 22 %) at 12 months and in females (by 16 %) from 38 mg/kg bw/d at 24 months. At the latter time-point, the relative liver weight was increased by 7 % in males and 23 % in females at 163 / 302 mg/kg bw/d mefentrifluconazole.

There were no treatment-related findings upon gross necropsy at the 12- or 24-month intervals. At 12 months, a minimal or slight treatment-related hepatocellular centrilobular hypertrophy was observed in the high-dose group (191 / 300 mg/kg bw/d in males / females) in 6 / 10 males and 5 / 10 females. Histopathology at 24 months revealed statistically significant increases in the incidences of non-neoplastic changes in the liver. Given the increases in relative weights, the findings in the liver (minimal centrilobular hypertrophy in 15 / 50 males and 7 / 50 females at 163 / 302 mg/kg bw/d) are concluded by the dossier submitter to be treatment related. There were no other dose-related changes in non-neoplastic histopathology findings.

In an 18-month mouse carcinogenicity study, there were no overt clinical signs of toxicity in any test group or an effect on survival. Treatment-related reductions in body weight and body-weight gain occurred in males at 36 mg/kg bw/d from week 11 onwards and in females at 61.5 mg/kg bw/d from week 7 onwards; at the mid-dose of 12.6 mg/kg bw/d, body weights and body-weight gains of females were reduced from week 34. Gross

necropsy did not reveal any treatment-related changes. Relative liver weights were increased at all doses in males (by 12 %, 18 %, 42 % at 3.5, 9.1, 36 mg/kg bw/d) and at the high-dose level in females (by 57 %). Upon histopathology, a diffuse fatty change of hepatocytes was observed in the liver of most animals. Although there was no difference in total incidence of this change compared with the control animals, the severity was slightly increased in males treated at 9.1 and 36 mg/kg bw/d and in females at 61.5 mg/kg bw/d. In the same dose groups, the incidence and severity of macrovesicular fatty change was increased; this finding was characterised by the presence of large vesicles within the hepatocytes. In addition to the fatty changes, eosinophilic cytoplasmic inclusions in hepatocytes (centrilobular distribution) were recorded in the majority of the males at 36 mg/kg bw/d and hepatocellular single cell necrosis (minimal severity) occurred in 20 % of the high-dose females (61.5 mg/kg bw/d).

A dose-related decrease in absolute kidney weights was reported only in males; when this was adjusted for body weight, only the change at 36 mg/kg bw/d was statistically significant. However, histopathology changes (decreased incidence of tubular vacuolation) were observed in the kidneys of males of the mid- and high-dose groups; these were likely to be an expression of increased (energy-consuming) excretion activity and were not indicative of a degenerative process. Adrenal-gland weights were increased in the high-dose males (absolute and relative) and females (absolute). In males, there were no associated macroscopic or microscopic findings. In females, there were histopathology changes at 61.5 mg/kg bw/d, which consisted of an increased incidence of eosinophilic cytoplasmic change and increase in the size of individual eosinophilic cells. No signs of degenerative processes were observed in conjunction with this finding. In the thyroid, the incidence of follicular-cell hyperplasia of males at 36 mg/kg bw/d and of females at 61.5 mg/kg bw/d was increased.

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

The repeated-dose oral toxicity of mefentrifluconazole has been investigated in 28-day and 90-day studies in rats, mice and dogs, a one-year study in dogs and in chronic / carcinogenicity studies in rats and mice. Its dermal repeated-dose toxicity has been investigated in a 28-day study in rats. No adverse effects were reported in the dermal study when mefentrifluconazole was applied at doses up to 1000 mg/kg bw/d.

Following oral administration from 28 days to one year, the liver was a clear target organ in all species. Effects on the liver comprised treatment-related increases in absolute and relative weights, clinical chemistry alterations and histopathology findings. Impairment of liver function was observed from 256 mg/kg bw/d in rats and 150 mg/kg bw/d in dogs, whilst clear liver toxicity (including liver foci and hepatocellular necrosis) was reported from 47.9 mg/kg bw/d in mice. The severity of the liver effects did not markedly increase with an increase in exposure duration from 28 to 90 days, or to one year in dogs. Following administration of mefentrifluconazole to rats for 12 (doses up to 191 / 300 mg/kg bw/d in males / females) and 24 months (doses up to 163 / 302 mg/kg bw/ in males / females), adaptive changes of the liver were indicated by increased relative weights and minimal-to-slight hepatocellular hypertrophy. In the 18-month mouse study, an increase in the severity and/or incidence of hepatocellular fatty change was evident from 9.1 mg/kg bw/d in males and at 61.5 mg/kg bw/d in females. 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/d). The results of this study gave no indication that the liver toxicity obviously worsened 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.

Based upon the dose levels at which liver impairment / toxicity was evident and also the nature of the effects compared with those in rats and dogs, the mouse was the most sensitive species to this effect.

Another consistent finding across species was a reduction in body weight and body-weight gain; this wasn't always attributable to a lack of palatability, because it also occurred when mefentrifluconazole was administered in capsules. This effect was the most sensitive indicator of toxicity in rats and dogs, whilst histopathological indicators of hepatocellular toxicity were the most sensitive indicators in mice.

Other findings were not consistent across species and/or studies. Haemoconcentration was noted in the 90-day mouse study at a dose (58 / 67 mg/kg bw/d) below the guidance cut-off value for classification, but 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 (section 10.9), the dossier submitter concludes that they are not evidence of an adverse event. Slight changes in haematology parameters were noted in rats following 12 months of mefentrifluconazole administration from 34 mg/kg bw/d but were not consistent with haemoconcentration.

Some histopathology changes were noted in mice following 18 months of administration in the kidney (from 9.1 mg/kg bw/d in males), adrenal glands (at 61.5 mg/kg bw/d in females) and thyroid (at 36 / 61.5 mg/kg bw/d in males and females). These 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.

10.12.2 Comparison with the CLP criteria

STOT-RE is assigned on the basis of a substance demonstrating evidence of significant or severe toxicity, generally at or below the oral guidance value of 100 mg/kg/d (for a classification in category 2) obtained in a 90-day rat study. The oral guidance value for a classification in category 1 is ≤ 10 mg/kg/d. The equivalent guidance values for a 28-day study are ≤ 300 mg/kg/d and ≤ 30 mg/kg/d, respectively; for a one-year study, they are ≤ 25 mg/kg/d and 2.5 mg/kg/d, respectively, and for a two-year study, ≤ 12.5 mg/kg/d and 1.25 mg/kg/d. For dermal exposure, the 90-day guidance value is ≤ 200 mg/kg/d in rats or rabbits. 'Significant' toxicity is taken to mean changes that clearly indicate functional disturbance or morphological changes that are toxicologically relevant. 'Severe' toxicity is considered to be more profound or serious and indicates changes that are of a considerably adverse nature with a significant impact on health.

No adverse effects were recorded when mefentrifluconazole was administered dermally to rats at the limit dose of 1000 mg/kg bw/d. In several oral repeated-dose toxicity studies in rats, mice and dogs, the only clear target organ was the liver. The effects reported in these oral studies at doses below the guidance cut-off values for STOT-RE are summarised below.

Table 49. Comparison of the guidance values for STOT-RE against the effects in the mefentrifluconazole repeated-dose toxicity studies

Study	(Adjusted) guidance value category 1 / 2 (mg/kg bw/d)	Effects at doses below guidance cut-off values
28-d rat study	30 / 300	Category 1: Lowest dose = 47 mg/kg bw/d Category 2: No adverse effects at 47 mg/kg bw/d & 135 / 138 mg/kg bw/d
28-d mouse study	30 / 300	Category 1: ≤ 15.5 / 18.5 mg/kg bw/d = increased relative liver weight & hepatocellular hypertrophy (adaptive) Category 2: ≥ 47.9 / 61 mg/kg bw/d = increased relative liver weight & hepatocellular hypertrophy (adaptive) 128 / 145 mg/kg bw/d = 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/d Category 2: 125 – 250 mg/kg bw/d = 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/d (no adverse effects) Category 2: No adverse effects at 76 / 91 mg/kg bw/d

90-day mouse study	10 / 100	Category 1: No adverse effects at 2 /3 mg/kg bw/d Category 2: ≥ 11 / 15 mg/kg bw/d = haemoconcentration, clinical chemistry changes 58 / 67 mg/kg bw/d = increased liver weight, liver single-cell necrosis (grade 1, 2/10 males) & cytoplasmic alteration (grade 1, 4/10 males)
90-d dog study	10 / 100	Category 1: Lowest dose = 15 mg/kg bw/d (no adverse effects) Category 2: No adverse effects at 90 mg/kg bw/d
1-year dog study	2.5 / 25	Category 1: Lowest dose = 10 mg/kg bw/d Category 2: 10 mg/kg bw/d = minimal eosinophilic change (3/5 males, 2/3 females) – adaptive change; no adverse effects
2-year rat study	2.5 / 25 (one-year) 1.25 / 12.5 (two-year)	Category 1: Lowest dose = 4 / 6 mg/kg bw/d (no adverse effects at two years) Category 2: 31 / 41 mg/kg bw/d (12 months) = slight haematology & clinical-chemistry changes 25 / 38 mg/kg bw/d (two years) = increased relative liver weight in females
18-month mouse study	1.7 / 17	Category 1: Lowest dose = 3.5 / 4.9 mg/kg bw/d (no adverse effects) Category 2: 9.1 / 12.6 mg/kg bw/d = 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).

In rats and dogs, no adverse effects were reported at doses below the guidance cut-off values for category 1. In mice, increased liver weight and hepatocellular hypertrophy were observed at doses below the guidance cut-off value in the 28-day study, which were indicative of an adaptive rather than an adverse effect. No adverse effects below the guidance value for category 1 occurred in mice when the substance was administered for 90 days or 18 months. Therefore, mefentrifluconazole does not meet the criteria for classification in category 1.

In rats, no adverse effects below the guidance cut-off for category 2 occurred in 28-day or 90-day studies. Upon prolonged administration, there were slight haematology and clinical-chemistry changes at 12 months and increased liver weight, albeit without histopathology changes. In dogs, effects below the cut-off value for category 2 were observed in the range-finding 28-day study and the one-year study. These effects in either or both studies comprised decreased body weight, increased liver weight with hepatocellular hypertrophy, eosinophilic change of hepatocytes and delayed food intake. The observed eosinophilic change was of minimal to slight severity and was not a clear indicator of hepatotoxicity. None of these effects was reproduced in the 90-day study at doses below the cut-off value. Overall, considering the adaptive nature of the liver effects (with the eosinophilic change being of only slight severity) and the small numbers of animals investigated, the dossier submitter concludes that these changes in dogs do not warrant classification.

In mice, liver effects were noted at doses below the guidance cut-off value for category 2 in all studies. These effects comprised hepatocellular hypertrophy, necrosis, oval-cell proliferation, bile-duct hyperplasia, cytoplasmic alteration and fatty change. Additionally, haemoconcentration and clinical chemistry changes

occurred following 90 days of administration, but not after 18 months. These findings, and those in the rat chronic / carcinogenicity study, are compared with the classification criteria below.

In accordance with the guidance on the application of the CLP criteria, the following effects might be indicative of significant or severe toxicity and thus merit classification for STOT-RE.

a) Morbidity or death resulting from repeated or long-term exposure.

Morbidity and death were not features of exposure to mefentrifluconazole.

b) Significant functional changes in the central or peripheral nervous systems or other organ systems

This was not a feature of exposure to mefentrifluconazole.

c) Any consistent and significant adverse change in clinical biochemistry, haematology or urinalysis parameters

Following 12 months of mefentrifluconazole administration to rats, slight haematology and clinical chemistry changes were noted that consisted of a 5 % reduction in activated partial thromboplastin time in males. At a dose relevant for classification in category 2, the only clinical-chemistry changes were increases in ALP (39 %) and urea (17 %), again only in males. The changes in ALT levels were relatively small and were probably an indication of liver-enzyme induction as a result of an adaptive response rather than adversity. Other changes that were observed at higher doses, such as lower glucose levels, perhaps reflected increased energy consumption as a result of greater liver-cell metabolism. Overall, 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 reduced PTT indicated an increased synthesis of coagulation factors in the liver. The dossier submitter thus concludes that these effects in rats were treatment related but not adverse and do not support classification.

In mice, haemoconcentration was reported in the 90-day study in males only, but not in the 28-day or 18-month studies. Since the effects on haemoglobin and haematocrit constituted changes of ≤ 5 %, they did not provide convincing evidence of toxicity. The haemoconcentration possibly resulted from decreased water consumption, since there was no histological evidence that either of the other likely explanations (a stress-related response or an increased production of red blood cells) were responsible. Clinical chemistry changes comprised reductions in cholesterol; in isolation, these are not regarded by the dossier submitter to be adverse.

d) Significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination

Gross necropsy and histological examination did not reveal any indications of significant organ damage.

e) Multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity

Hepatocellular necrosis was observed in mice in the 28-day and 90-day studies at doses below the guidance cut-off value for category 2. In the 28-day study, the necrosis was multifocal and of minimal/slight severity. The necrosis that occurred after 90 days' administration was single cell, of minimal severity and in only 20 % of males, with no cases in females. Necrosis was not observed upon a longer duration of exposure (18 months).

Given that the multi-focal necrosis was only observed in the 28-day range-finding study, was of minimal to slight severity, and was not observed upon longer durations of exposure, this finding does not constitute evidence of significant or severe toxicity and thus does not support classification.

f) Morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction (e.g., severe fatty change in liver)

A slight increase in the severity of hepatocellular fatty change was recorded in male mice after 18 months of exposure at a dose below the guidance cut-off for category 2. This change was not seen after shorter exposure durations. Since almost all males, whatever the group, had at least slight (grade 2) diffuse fatty change, the increase in severity grade to 2.9 (verging upon moderate) at 9.1 mg/kg bw/d mefentrifluconazole probably

reflects a slight exacerbation of age-related hepatocellular changes. The incidence of macrovesicular fatty change was also high in the control animals (approaching 50 %), but in this case there was a statistically significant increase in incidence in the 9.1 mg/kg bw/d group (to 70 %) together with a slight increase in severity (from 0.5 in controls to 1.5 (minimal / slight) in the exposed group). Considering that there was a high incidence of this finding in the controls, the changes in incidence and severity might, again, have represented a slight exacerbation of age-related pathology; the absence of fatty change in any group in the shorter-duration studies would support this view. In addition to fatty change, minimal-grade oval-cell proliferation and bile-duct hyperplasia were observed in the 28-day mouse study at doses below the guidance cut-off values for category 2, but not upon longer duration even at doses that exceeded the guidance value.

The criteria specify that morphological changes should provide clear evidence of marked organ dysfunction. The dossier submitter considers that marked liver dysfunction was not demonstrated with mefentrifluconazole, since the fatty changes were scored as less than moderate. Furthermore, apart from an increase in liver weight, which might have been at least partially an adaptive change, there were no other indications of liver dysfunction. Long-term survival of the animals (to 18 months) was not affected.

g) Evidence of appreciable cell death in vital organs incapable of regeneration

This was not a feature of exposure to mefentrifluconazole in any of the studies.

In conclusion, the only target organ at doses below the guidance cut-off value for category 2 was the liver. Based on the nature of the effects and the doses at which these occurred, the mouse was more sensitive than the rat and dog. This is consistent with the usual perception that mice tend to be particularly susceptible to liver toxicity.

Overall, the dossier submitter concludes that the necrosis and morphological changes in the liver were not sufficiently severe or reproducible to warrant classification.

10.12.3 Conclusion on classification and labelling for STOT RE

Not classified (conclusive but not sufficient for classification).

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)
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.

Adverse change in haematology and clinical chemistry in mice

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.

Multi-focal or diffuse necrosis, fibrosis or granuloma formation in mice

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.

Supplemental information - In depth analyses by RAC

During the assessment of the STOT RE, the data obtained from three dose range-finding studies on rabbits were also assessed. The detailed description of these studies can be found in the reproductive toxicity section. Since these data were evaluated for acute toxicity oral as well, specific comments can be found in the corresponding section.

As in the case of the acute toxicity, RAC considers that the toxicity shown in these studies are specific to rabbits and not relevant to humans. Consequently, the findings were not taken into account in the assessment of the STOT RE.

10.13 Aspiration hazard

Not relevant for solid substances.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

Table 50: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
Ready biodegradation OECD Guideline 301B, GLP	Not readily biodegradable - No degradation after 28 days at 22°C	Valid	Schwarz, 2014a (section B.8.2.2.1 in the DAR)
Aquatic hydrolysis OECD Guideline 111, GLP	Stable (<10% degradation) at pH 4, 5, 7 and 9 at 25°C	Valid	Hassink, 2015b (section B.8.2.1.1 in the DAR)
Aerobic mineralization OECG Guideline 309, GLP	Not significantly degraded - <10% Applied Radioactivity mineralised after 63 days at 20°C	Valid	Michel, 2015a (section B.8.2.2.2 in the DAR)
Water/sediment simulation OECD Guideline 308, GLP	DT_{50} of 122.2 to 213.1 days (20^oC) based on whole system. Mineralisation: minimal with <10% Applied Radioactivity	Valid	Ebert and Dalkmann, 2015a (section B.8.2.2.3 in the DAR)
Aquatic photolysis OECD Guideline 316, GLP	DT_{50} of BAS 750 F of 2.3 days	Valid	Zhixing, 2015a (section B.8.2.1.2 in the DAR)

11.1.1 Ready biodegradability

A ready biodegradation study (Schwarz, 2014a) is available following OECD Guideline 301B (CO_2 Evolution (Modified Sturm Test)) and GLP. Municipal activated sludge was collected and four differing mineral mediums were prepared. The mineral mediums were adjusted to pH 7.4 (if necessary) and mefentrifluconazole applied; the samples were stored at 22°C. No biodegradation of the test substance was observed in the 28 day period, therefore, the substance is considered not readily biodegradable.

11.1.2 Hydrolysis

An aqueous hydrolysis study (Hassink, 2015b) is available following GLP and OECD Guideline 111. Mefentrifluconazole was applied to buffer solutions of pH 4, 5, 7 and 9 and stored at 25°C for 30 days in the dark. No significant degradation was observed with analysis showing less than 10% degradation after 30 days. On this basis, mefentrifluconazole is considered hydrolytically stable.

11.1.3 Other convincing scientific evidence

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

An aerobic mineralisation study (Michel, 2015a) is available following GLP and OECD Guideline 309. Natural surface water (physico-chemical properties: pH 7.2, temperature 13.6°C at sampling site, DOC 3.8 mg/L) was collected and mefentrifluconazole was applied at concentrations of 10 µg/L and 100 µg/L; the samples were incubated at 20 ± 1°C. No significant degradation was observed with analysis showing less than 10% degradation, at both test concentrations, after 63 days.

An aerobic water/sediment degradation study (Ebert and Dalkmann, 2015a) is available following OECD Guideline 308 and GLP. Two natural water/sediment systems of differing organic carbon amounts were sampled in November 2013 for the chlorophenyl and triazole radiolabelled experiments and in May 2014 for the trifluoromethylphenyl radiolabelled experiment. The physico-chemical properties of the two systems are summarised as follows:

Table 51: Summary of water and sediment properties from OECD 308 study on mefentrifluconazole

Water			**System Berghäuser Altrhein**		**System Ranschgraben**	
Field sampling			**November 2013**	**May 2014**	**November 2013**	**May 2014**
Temperature	[°C]		9.1	22.9	6.7	16.6
pH			7.4	8.40	7.30	7.10
Redox potential	[mV]		281	263	273	219
TOC / org. C	[mg L^{-1}]	Beginning	5.6	3.0	6.0	4.6
		End	10.0	6.7	6.1	4.7
Sediment			**System Berghäuser Altrhein**		**System Ranschgraben**	
Sampling depth*	[cm]		0 - 20	0 – 10	0 - 10	0 - 10
pH	($CaCl_2$)		7.1	7.0	5.2	6.0
Redox potential*	[mV]		-134	-407	-402	-322
TOC / org. C	[%]	Beginning	1.80	6.27	2.94	2.00
		End	1.79	6.10	2.79	1.72
Soil type (USDA classification			silty loam	silty clay loam	fine sand	fine sand

The study was conducted at 20°C, in the dark. The test substance was applied to the water phase but was found to partition to sediment; after 100 days < 5% Applied Radioactivity (AR) was detected as not degraded parent compound in the water phase with > 45% AR BAS 750 F detected in the sediment phase of both systems. This is not unsurprising given its relatively high K_{FOC} value of 3455.6 mL/g (see section 11.3.1.). 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 (geomean 163.4 days).

11.1.3.2 Photochemical degradation

An aqueous photolysis study (Zhixing, 2015a) is available following GLP and OECD Guideline 316. Test solutions were incubated at pH 7 for 15 days at 25°C under constant irradiation. 'Suntest' apparatus with a xenon lamp was used and a filter system to absorb UV radiation below 290 nm. The average light intensity was 571 W/m^2, equivalent to natural sunlight at 40°N latitude. Rapid photolysis of mefentrifluconazole was observed, 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) which were assigned the following codes: M750F005, M750F006, M750F007 and M750F008. Mefentrifluconazole was calculated as having a photolytic DT_{50} of 2.3 days.

Summary and discussion of degradation

Mefentrifluconazole is considered hydrolytically stable and not readily biodegradable. Furthermore, no significant degradation in the aerobic mineralisation study was observed.

Mefentrifluconazole was observed to degrade rapidly in the aqueous photolysis study with a number of degradants forming. A DT_{50} value of 2.3 days was calculated for mefentrifluconazole by SFO kinetics. Photolysis is of uncertain relevance as a route of degradation in typical European aquatic environments and, given the available data, there is insufficient information in this case to evaluate photodegradation in terms of mineralisation or transformation to non-classifiable substances. Therefore, aquatic photolysis is not considered further in relation meeting the criteria for rapid degradation.

In an aerobic water/sediment study, mefentrifluconazole was observed to dissipate rapidly from the water phase to the sediment phase (water geomean $DissT_{50}$ 1.5 days, $DissT_{90}$ 24.0 days). A maximum of 5.1% mineralisation to CO_2 was observed after 100 days. Total system $DegT_{50}$ values of 122.2 and 213.1 days were calculated by SFO kinetics, with a geomean value of 163.4 days (at 20 ^{o}C).

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

11.2 Environmental transformation of metals or inorganic metals compounds

Not applicable.

11.3 Environmental fate and other relevant information

11.3.1 Adsorption/Desorption

One GLP study (Vasques, 2015a, section B.8.1.2.1 of the DAR) is available investigating the adsorption of mefentrifluconazole. The study followed OECD Guideline 106 and used 8 soils with % organic carbon ranging from 0.6 – 3.4. The K_{FOC} values ranged between 2010 and 4931 mL/g with a geomean K_{FOC} value of 3455.6 mL/g. As indicated by the water-sediment study (see section 11.1.3.1) this relatively high K_{FOC} data indicates a propensity for mefentrifluconazole to dissipate from the water column into sediment. In soil, these results would indicate that mefentrifluconazole has only low or slight mobility.

11.3.2 Volatilisation

Physico-chemical properties of mefentrifluconazole are summarised in Table 8, Section 7. Experimental data (Kroehl, 2014a) indicates the vapour pressure for mefentrifluconazole is 3.2×10^{-6} Pa at 20°C based on following OECD Test Guideline 104. The Henry's Law Constant (Kroehl,

2014c) was calculated to be 1.6 x 10^{-3} Pa m^3 mol^{-1} at 20 °C, indicating mefentrifluconazole is unlikely to partition from the water phase to air.

11.4 Bioaccumulation

Table 52: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
Partition coefficient *n*-octanol/water Calculation based on solubility in water and *n*-octanol	Results determined at 20 °C applying the HPLC method. pH 4*: log K_{ow} = 3.4 pH 7: log K_{ow} = 3.4 pH 7*: log K_{ow} = 3.3 pH 9*: log K_{ow} = 3.4 * buffered	Valid Log K_{ow} is not pH dependent	Wilbrand, 2013c
Experimental aquatic BCF OECD Guideline 305, GLP	Steady state whole fish lipid-normalized kinetic BCF_{KLg}: 385 L/kg $t1/2_g$ (growth-corrected depuration half-life): 0.60 day	Flow through, 14 days exposure, 7 days depuration	[redacted] 2015c

11.4.1 Estimated bioaccumulation

As relevant experimental data are available, estimations are not included.

11.4.2 Measured partition coefficient and bioaccumulation test data

An experimental aquatic study to determine the bioconcentration potential (BCF) of mefentrifluconazole (purity 98.8%) is available following GLP and OECD Guideline 305 ([redacted] 2015c). The study used a mixture of radiolabelled ^{14}C- mefentrifluconazole and unlabelled test substance (ratio 2:1), a flow-through system with Rainbow Trout (*Oncorhynchus mykiss*) and exposure to a single concentration of test substance at 10 µg/L (0.010 mg/L) Additionally, a water control was set up.. The exposure period ran for 14 days followed by a 7-day depuration period. Test substance concentrations in water and fish as well as wet weight of fish were determined throughout the study. Mortality and signs of toxicity were assessed daily.

No mortalities or signs of toxicity were observed in the control and treatment group over the 23 day test period. There was no statistically significant difference in fish growth rate between control and treatment group during the experiment, therefore data from both groups were combined to determine the overall growth rate (kg) for "growth-corrected" calculations. The lipid content of control fish sampled over the test period remained constant considering the variability of individual values and the mean lipid content from the uptake period (2.1%) was used for lipid normalization calculations.

The concentration in fish reached 95% steady state within 2.6 days based on the kinetic calculations. Overall the measured steady state bioconcentration factor (BCF_{ss}) values were very similar to the calculated kinetic (BCF_K) values indicating that steady state was reached and that uptake and depuration follow first order kinetics. The accumulation in the edible fish portions was less than in

the non-edible portions. However, the most relevant BCF is the growth corrected kinetic BCF normalized to 5% lipid content (BCF_{KLg}) for the whole fish because it incorporates all measurements during uptake and depuration and since it removes the influence of the test fish lipid content. This whole fish BCF_{KLg} was determined to be 385 based on total radioactive residues of mefentrifluconazole.

Summary and discussion of aquatic bioaccumulation

The log K_{ow} value of 3.4 for mefentrifluconazole is below the CLP log K_{ow} trigger value of ≥ 4 intended to identify substances with a potential to bioaccumulate under CLP. Nevertheless, an experimental bioconcentration study in fish is available to consider bioaccumulation further.

In the experimental study, whole fish BCF values for mefentrifluconazole were less than 500 indicating a low potential for bioaccumulation. Additionally, rapid depuration of mefentrifluconazole was observed with a depuration $t1/2_g$ of 0.60 days (based on total radioactivity). To conclude, the substance does not meet CLP criteria as a bioaccumulative substance.

11.5 Acute aquatic hazard

A summary of the suitable acute aquatic toxicity studies for mefentrifluconazole, as reviewed under EU Regulation 1107/2009, is presented in Table 53 below. The studies have been evaluated, considered reliable and deemed suitable for hazard classification purposes. All studies below conformed to GLP certification and were valid according to the criteria of the respective test guidelines. Additional information on the studies supporting mefentrifluconazole has been presented in the subsections below.

Although valid and reliable studies are available on the formulated product “BAS 750 01 F” containing 100.0 g BAS 750 01 F/L, studies using technical mefentrifluconazole on the same species and endpoints are available which should be used in preference as co-formulants could affect interpretation of the substance classification. Therefore the studies using mefentrifluconazole will take precedence and those using “BAS 750 01 F” have not been included below.

A number of studies have used loaded filtrate columns of mefentrifluconazole and sequential dilutions of this filtrate rather than use nominal based dissolved solutions. This is to ensure that no precipitate forms over the duration of these studies, as mefentrifluconazole is near or at the limit of solubility in these studies for mefentrifluconazole of 0.71mg/L (pH 7, phosphate buffer).

Table 53: Summary of relevant information on acute aquatic toxicity

Guideline	Species	Endpoint Data	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity (mg/L)[2] [data endpoint is based upon[3]]	
Fish							
OECD 203 (1992)	*Oncorhynchus mykiss*	Mortality	Flow through	96h	LC_{50}	**0.532 mm**	[redacted] (2014)
OECD 203 (1992)	*Danio rerio*	Mortality	Static	96h	LC_{50}	0.906 mm	[redacted] (2015)
OECD 203 (1992)	*Cyprinodon variegatus*	Mortality	Semi-static	96h	LC_{50}	0.761 mm	[redacted] (2014)
OECD 203 (1992)	*Cyprinus carpio*	Mortality	Flow through	96h	LC_{50}	1.126 mm	[redacted] (2015c)
Aquatic invertebrates							
OECD 202 (2004)	*Daphnia magna*	Immobility	Static	48h	EC_{50}	**0.944 mm**	Brzozowska (2014a)
EPA 850.1035	*Americamysis bahia*	Mortality	Flow through	96h	LC_{50}	1.3 mm	VanHooser (2014a)
EPA 850.1025	*Crassostrea virginic*	Shell growth inhibition, mortality	Flow through	96h	EC_{50}	0.9472[1] mm [shell growth inhibition]	VanHooser (2015a)
Algae / aquatic plants							
OECD 201 (2011)	*Pseudokirchneriella subcapitata*	Growth rate and morphology	Static	72h	E_rC_{50}	1.352 mm	Brzozowska (2014b)
				72h	E_rC_{10} NOE_rC	0.904 mm 0.209 mm	
EPA 850.4500	*Skeletonema costatum*	Growth rate	Static	72h	E_rC_{50}	**0.679 mm**	Bergfield (2015a)
				72h	E_rC_{10} NOE_rC	0.373 mm 0.0985 mm	
EPA 850.4500	*Navicula pelliculosa*	Growth rate	Static	72h	E_rC_{50}	1.347 mm	Bergfield (2015b)
				72h	E_rC_{10} NOE_rC	0.478 mm 0.303 mm	
EPA 850.4500	*Anabaena flos-aquae*	Growth rate	Static	72h	E_rC_{50}	> 3.08 mm	Bergfield (2015c)
				72h	E_rC_{10} NOE_rC	> 3.08 mm ≥ 3.08 mm	
OECD 221 (2006)	*Lemna gibba*	Growth rate	Static	7d	E_rC_{50}	**> 2.017 im**	Swierkot (2014a)
					E_rC_{10} NOE_rC	> 2.017 im ≥ 2.017 im	

[1]Study endpoint based upon shell growth

[2] Endpoints in bold are the critical endpoints for that organism group. The letters to the right of the numbers refer to what concentration of the active substance the endpoint is based on:
n – nominal mm – mean measured im – initial measured

[3]Where multiple types of data capable of producing the stated endpoints have been recorded

11.5.1 Acute (short-term) toxicity to fish

Four acute fish studies were submitted and all were to OECD 203 (1992), according to GLP and considered acceptable.

[redacted]., 2014a

In a 96-hour flow-through acute toxicity laboratory study, juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to a dilution water control and to mean measured concentrations of < LOQ, 0.069, 0.142, 0.380, 0.826 and 1.55 mg a.s./L (on which the biological results are based) in groups of 10 animals in glass aquaria containing 9L water. Fish were observed for survival and toxicity within 1 and at 6, 24, 48, 72 and 96 hours. After 96 hours, no mortality was observed in the control and at test substance concentrations of up to and including 0.142 mg a.s./L, whereas 5% mortality was observed at 0.380 mg a.s./L. At the two highest tested concentrations, all fish were dead after 96 hours of exposure. Sub-lethal effects were observed at 0.380 mg a.s./L after 96 hours. The 96-hour mean measured LC_{50} was 0.532 mg a.s/L.

[redacted], 2015a

In a 96-hour static acute toxicity laboratory study, juvenile zebrafish (*Danio rerio*) were exposed to a reconstituted water control and filtrate dilutions of 10 mg mefentrifluconazole/L corresponding to geometric mean concentrations of 1.110, 0.913, 0.735, 0.593 and 0.475 mg a.s./L, respectively, on which the biological results are based. Fish were kept in groups of 10 animals in 10 L glass aquaria and were observed for survival and toxicity at 3, 6, 24, 48, 72 and 96 hours. After 96 hours of exposure, no mortality was observed in the control and at test substance concentrations of up to and including 0.593 mg a.s./L, whereas 5% and 45% mortality was observed at 0.735 and 0.913 mg a.s./L, respectively. At the highest tested concentration, all fish were dead. Sub-lethal effects were observed at 0.735 and 0.913 mg a.s./L after 96 hours. The 96-hour mean measured LC_{50} was 0.906 mg a.s/L.

[redacted]., 2014 a

In a 96-hour semi-static acute toxicity laboratory study, juvenile sheepshead minnow (*Cyprinodon variegatus*) were exposed to a water control, a solvent control and to nominal concentrations of 0.18, 0.35, 0.70, 1.4 and 2.7 mg mefentrifluconazole/L in groups of 10 animals in glass aquaria containing 8 L water. These corresponded to mean measured concentrations of 0.131, 0.258, 0.517, 1.12 and 2.05 mg a.s./L, on which the biological results are based. Fish were observed for survival and toxicity at 6, 24, 48, 72 and 96 hours. After 96 hours, no mortality was observed in the water control and the solvent control and the two higher concentrations of 0.258 and 0.517 mg a.s./L. Mortality at 5% occurred at the lower 0.131 mg a.s./L, but was not considered biologically significant. All fish were dead at the two highest test substance concentrations after 96 hours of exposure. Sublethal effects were observed at 0.258 and 0.517 mg a.s./L after 96 hours. The 96-hour mean measured LC_{50} was 0.761 mg a.s/L.

[redacted]., 2015 c

In a 96-hour flow-through acute toxicity laboratory study, common carp (*Cyprinus carpio*) were exposed to a dilution water control and to nominal concentrations of 4.6, 10, 22, 46 and 100% of a saturated solution of mefentrifluconazole in groups of 10 animals in glass aquaria containing 9 L water. These corresponded to mean measured concentrations of 0.082, 0.171, 0.414, 0.812 and 1.57 mg a.s./L on which the biological results are based. Fish were observed for survival and symptoms of toxicity within 1 and at 6, 24, 48, 72 and 96 hours after start of exposure. After 96 hours, no mortality was observed in the control and at test substance concentrations of up to and including 0.812 mg a.s./L, whereas 100% mortality was observed at 1.57 mg a.s./L. No sub-lethal effects were observed

in the control and at test substance concentrations of up to and including 0.812 mg a.s./L. The 96-hour mean measured LC_{50} was 1.126 mg a.s/L.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

Three acute aquatic studies were submitted, all according to GLP and considered acceptable. However, one of the studies was performed on eastern oysters and the EC_{50} was based on shell deposition rather than mortality or immobility. Consequently, it has not been given the same weighting as the other studies.

Brzozowska K., 2014a

In a 48-hour static study performed to OECD 202 (2004), the effect of mefentrifluconazole on water flea (*Daphnia magna*) neonates was investigated. A filtrate of the loading of 10 mg mefentrifluconazole/L was used as the highest test substance concentration. Six lower test substance concentrations consisted of dilutions of this filtrate, equivalent to geometric mean measured concentrations of 0.156, 0.254, 0.373, 0.591, 0.838, 1.225 and 1.854 mg mefentrifluconazole/L, upon which the results are based. A dilution water control was set up. Daphnids were exposed in 4 replicates per concentration, containing 5 daphnids each. The daphnids were observed for immobility after 24 and 48 hours. After 48 h of exposure, no immobility of daphnids was observed in the control and at test substance concentrations of up to and including 0.254 mg a.s./L. Immobility at 20%, 30%, 45%, 65% and 75% was observed at the test substance concentrations of 0.373, 0.591, 0.838, 1.225 and 1.854 mg a.s./L, respectively. Statistically significant effects on mobility of daphnids were detected at the five highest test substance concentrations. The 48-hour mean measured EC_{50} was 0.944 mg a.s/L.

VanHooser A., 2014a

In a 96-hour flow-through acute toxicity laboratory study according to EPA 850.1035, saltwater mysids (*Americamysis bahia*) were exposed to a dilution water control, a solvent control and to mean measured concentrations of 0.227, 0.415, 0.896, 1.76 and 3.29 mg a.s./L (upon which the results are based) in two replicates per treatment containing 10 mysids each. Saltwater mysids were observed for survival and symptoms of toxicity 24, 48, 72 and 96 hours after start of exposure. After 48 hours of exposure no mortality and no other toxic effects were observed in the control, the solvent control and at test substance concentrations of up to and including 0.896 mg a.s./L, whereas 70% mortality and sub-lethal effects were observed at 1.76 mg a.s./L and 100% mortality at 3.29 mg a.s./L. The 96-hour mean measured LC_{50} was 1.3 mg a.s/L.

VanHooser A., 2015a

In a 96-hour acute toxicity laboratory study the effect of mefentrifluconazole on shell deposition of eastern oysters (*Crassostrea virginica*) was investigated under flow-through conditions and performed to EPA 850.1025. Dosing was for water control, solvent control and test concentrations of 0.14, 0.25, 0.45, 0.80, 1.4 and 2.6mg a.s./L (nominal), corresponding to mean measured concentrations of 0.111, 0.174, 0.335, 0.623, 1.12 and 1.80mg a.s./L Eastern oysters were observed for survival and symptoms of toxicity daily during the 96h exposure period. Shell growth was statistically significantly inhibited at the three highest test substance concentrations compared to the control. The 96-hour mean measured EC_{50} was 0.947 mg a.s/L based on shell deposition.

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

Effects of the toxicity of mefentrifluconazole were tested on four species of algae (one to OECD 201 (2011) and three to EPA 850.4500) and one aquatic plant, *Lemna gibba* (OECD 221 (2006)). All were to GLP and considered acceptable.

Brzozowska K., 2014b

In a 96 hour static study conducted to OECD 201 (2011), the effect of mefentrifluconazole on the growth of the green alga *Pseudokirchneriella subcapitata* was investigated. A filtrate of the loading of 10 mg mefentrifluconazole/L was used as the highest test substance concentration with four dilutions of the filtrate, corresponding to geometric mean measured concentrations of 0.103, 0.209, 0.416, 0.914 and 1.899 mg mefentrifluconazole/L, upon which the results were based. Additionally, a dilution water control was set up. Assessment of growth was conducted daily. No morphological effects on algae were observed in the control and at test substance concentrations of up to and including 0.416 mg a.s./L. At 0.914 mg a.s./L about 15% and 20% of the cells were opalescent after 72 and 96 hours of exposure, respectively, while at 1.899 mg a.s./L about 20% of the cells were opalescent and 20% of the cells were comma-shaped after 72 and 96 hours of exposure. After 72 h of exposure, statistically significant effects compared to the control were detected at the four highest and at all test substance concentrations for growth rate and yield, respectively. The 72-hour mean measured E_rC_{50} was 1.352 mg a.s/L. The 72-hour mean measured E_rC_{10} was 0.904 mg a.s/L and the NOE_rC was 0.209 mg a.s/L.

Bergfield A., 2015a

The effect of mefentrifluconazole on the growth of the marine diatom *Skeletonema costatum* was investigated in a 96-hour static laboratory study conducted to EPA 850.4500. The following initial measured concentrations of 0.0490, 0.0985, 0.199, 0.419, and 0.845mg a.s./L, in addition to a water and vehicle control were tested. Effect concentrations were, however, subsequently derived based on mean measured concentrations over 72 hours. Four replicates were performed per concentration and control. Assessment of growth was conducted daily. After 72 h of exposure, statistically significant effects compared to the control were detected at the three highest test substance concentrations for growth rate and yield. Observations for any changes in the morphology of the algal cells were not performed. The 72-hour mean measured E_rC_{50} was 0.679 mg a.s/L. The 72-hour mean measured E_rC_{10} was 0.373 mg a.s/L and the NOE_rC was 0.0985 mg a.s/L.

Bergfield A., 2015b

In a 96-hour static acute toxicity laboratory study conducted to EPA 850.4500, the effect of mefentrifluconazole on the growth of the diatom *Navicula pelliculosa* was investigated. The following initial measured concentrations of 0.137, 0.303, 0.599, 1.30, 2.29, and 2.06 mg a.s./L and a water and solvent (dimethylformamide) control were tested. Effect concentrations were, however, subsequently derived based on mean measured concentrations over 72 hours. Assessment of growth was conducted daily. After 72 h and 96 h of exposure, statistically significant effects compared to the control were detected at test substance concentrations $\geq$ 0.724 mg a.s./L for growth rate and yield, respectively. Observations for any changes in the morphology of the algal cells were not performed. The 72-hour mean measured E_rC_{50} was 1.347 mg a.s/L. The 72-hour mean measured E_rC_{10} was 0.478 mg a.s/L and the NOE_rC was 0.303 mg a.s/L.

Bergfield A., 2015c

In a 96-hour static acute toxicity laboratory study conducted to EPA 850.4500, the effect of mefentrifluconazole on the growth of the freshwater blue-green alga *Anabaena flos-aquae* was

investigated. The following mean measured concentrations of 0.233, 0.476, 0.989, 1.85, and 3.08 mg a.s./L, in addition water and vehicle (dimethylformamide) controls were tested. Effect concentrations were, however, subsequently derived based on mean measured concentrations over 72 hours. Assessment of growth was conducted daily. After 72 hours of exposure, no statistically significant effects compared to the control were detected in any test concentration. Observations for any changes in the morphology of the algal cells were not performed. The 72-hour mean measured E_rC_{50} was >3.08 mg a.s/L. The 72-hour mean measured E_rC_{10} was >3.08 mg a.s/L and the NOE_rC was ≥3.08 mg a.s/L (the highest concentration tested).

Swierkot A., 2014a

In a 7-day static toxicity laboratory study, the effect of mefentrifluconazole on the growth of the duckweed *Lemna gibba* was investigated. A filtrate of the loading of 10 mg mefentrifluconazole/L was used as the highest test substance concentration with four dilutions of the filtrate, corresponding to initial measured concentrations of 0.119, 0.233, 0.485, 0.921 and 1.894 mg a.s./L, as concentrations were maintained within ±20% of initial measured concentrations at termination. Additionally, a dilution water control was set up. Assessment of growth and other effects were conducted 3, 5 and 7 days after test initiation. The percentage growth inhibition, relative to the control, was calculated for each test concentration based upon mean growth rates and final yield for the parameters frond number and dry weight. The duckweed population in the control vessels showed exponential growth, increasing 11-fold. The dry weight increased to an average of 22.4 mg per vessel in the control at test termination. No morphological effects on *Lemna* were observed for all test concentrations. No statistically significant differences compared to the control were observed at any test concentration for all measured parameters. The 7-day initial measured E_rC_{50} was >2.017 mg a.s/L. The 7-day initial measured E_rC_{10} was also >1.894 mg a.s/L and the NOE_rC was ≥2.017 mg a.s/L (the highest concentration tested).

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

Four studies on the effects of mefentrifluconazole were performed on sediment-dwelling invertebrates. One, on *Chironomus riparius*, was to OECD 218 (2004) and three to EPA 850.1735 (*Chironomus dilutes* and the marine amphipods *Hyalella azteca* and *Leptocheirus plumulosus*). All were to GLP and considered acceptable. However, in each of these studies, EC_{50} and NOEC values were only presented in relation to sediment concentrations of mefentrifluconazole (mg/kg). Since no mg/L endpoints are available, these studies will not be used for hazard classification.

11.6 Long-term aquatic hazard

A summary of the suitable aquatic toxicity studies for mefentrifluconazole, as reviewed under EU Regulation 1107/2009, is presented in Table 54 below. The studies have been evaluated, considered reliable and deemed suitable for hazard classification purposes. All studies below conformed to GLP certification and were valid according to the criteria of the respective test guidelines. Additional information on the studies supporting mefentrifluconazole have been presented in the subsections below. In line with the current CLP Guidance, preference is given to EC_{10} values for the chronic hazard classification over NOEC values and so EC_{10} values have been used where available.

Table 54: Summary of relevant information on chronic aquatic toxicity

Guideline	Species	Endpoint Data	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity (mg/L)[2] [data endpoint is based upon[3]]	
Fish							
Early life stage OECD 210 (2013)	*Danio rerio*	Hatchability, time to hatch and swim-up, survival, toxic signs and growth (body length)	Flow through	36d	NOEC[1]	**0.027 mm [growth (body length)]**	[redacted] (2015a)
Sexual development test OECD 234 (2011)	*Danio rerio*	Hatching success, survival, toxic signs, body weight, body length, maturity, sex ratio, gonad histopathology and vitellogenin.	Flow through	69d	NOEC[1]	≥ 0.045 mm	[redacted] (2015b)
Early life-stage EPA 850.1400	*Cyprinodon variegatus*	Hatchability, survival, toxic signs and growth	Flow through	35d	NOEC[1]	0.147mm [toxic signs]	[redacted] (2015)
Invertebrates							
OECD 211 (2012)	*Daphnia magna*	Parent mortality, reproduction (offspring per female), growth and population growth rate	Semi-static	21d	EC_{10} NOEC	**0.0175 n [reproduction (offspring per female)]** **0.010 n [reproduction (offspring per female)]**	Janson (2014a)
EPA 850.1350	*Americamysis bahia*	Survival, offspring per female, days to first brood release, length and dry weight	Flow through	28d	NOEC[1]	≥0.0132 mm	Dinehart (2016a)
OECD 211 (2012)	*Daphnia pulex*	Parent mortality, reproduction (offspring per female), growth and population growth rate	Semi-static	21d	EC_{10} NOEC	0.0573 n [reproduction (offspring per female)] 0.0282 n [reproduction (offspring per female)]	Janson (2015a)

OECD 211 (2012)	*Daphnia longispina*	Parent mortality, reproduction (offspring per female), growth and population growth rate	Semi-static	21d	EC_{10} NOEC	0.0558 n [reproduction (offspring per female)] 0.0338 n [reproduction (offspring per female)]	Janson (2015b)

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

[2] Endpoints in bold are the critical endpoints for that organism group. The letters to the right of the numbers refer to what concentration of the active substance the endpoint is based on:

n – nominal mm – mean measured im – initial measured

3 Where multiple types of data capable of producing the stated endpoints have been recorded

11.6.1 Chronic toxicity to fish

Three chronic fish studies were submitted all according to GLP and considered acceptable. Two were early life stage studies and one was a sexual development study in accordance with OECD 210 and OECD 234 respectively.

[redacted]., 2015 a

The study was a 35-day early life-stage test under flow-through conditions. Embryo sheepshead minnow (*Cyprinodon variegatus*) were exposed to a dilution water control, a vehicle control (0.05 mL DMF/L) and to mefentrifluconazole at nominal concentrations of 0.010, 0.020, 0.040, 0.080, and 0.16 mg a.s./L which corresponded to mean measured concentrations of 0.00861, 0.0172, 0.0356, 0.0725 and 0.147 mg a.s./L upon which the results were based. Hatchability, survival rate and behaviour of sheepshead minnow embryos and fry were assessed throughout the study. Individual fish lengths and weights were measured at test termination.

Egg hatch began on day 5 and ended between study days 6 and 11 in the control and all test substance treatments. The overall hatching success in the control and vehicle control was 91 and 93%, respectively. No statistically significant effects on the hatching success, post-hatch survival, time to start of hatch and time to end of hatch was observed for any of the test substance treatments as compared to the control. There was no statistically significant reduction in length and blotted wet weight in any of the test substance treatments as compared to the control.

The only morphological or behavioural abnormalities observed during the exposure were fish on the bottom of the test chamber and spinal curvature, but these abnormalities were judged not to be test substance related because no concentration-response relationship was evident. No other morphological or behavioural abnormalities were noted. The 35-day mean measured NOEC was 0.147 mg a.s/L based upon toxic signs observed in the test organisms.

[redacted]., 2015a

The chronic toxicity of mefentrifluconazole to zebrafish (*Danio rerio*) was evaluated in a 36-day early life-stage test under flow-through conditions. Embryos were exposed to a dilution water control and to nominal concentration of 0.010, 0.024, 0.060, 0.150 and 0.375 mg a.s./L, and mean measured concentrations of 0.011, 0.027, 0.063, 0.172 and 0.444 mg a.s./L, upon which the results are based. Hatchability, post-hatch survival rate, time to hatch and swim-up, and growth parameters of zebrafish embryos were assessed.

Hatching started simultaneously in all test groups on day 3 and was complete by day 6. No test substance-related effect was observed on the time to start or end of hatching. Hatching success ranged

from 96-100% for the control group. Survival from hatch to the end of swim-up (day 7) was 100% in the control group. There was no statistically significant decrease in hatching success or survival in any of the treatment groups.

In all test groups, larval swim-up started on day 5 of exposure and was complete simultaneously with the control on day 7. No test substance-related effect was observed on the time to swim-up. Survival was 96-100% for the replicates of the control group. The survival from the end of swim-up to the end of exposure (day 7-36) as well as the overall survival (day 0-36) was statistically significantly decreased in the treatment groups of 0.172 and 0.444 mg a.s./L.

There were no signs of toxicity or abnormalities observed among the replicates of the control group and the treatment groups. The total body lengths of the surviving fish at the end of the exposure period were statistically significantly decreased in the test groups of 0.063, 0.172 and 0.444 mg a.s./L. The mean wet weights of the surviving fish at the end of the exposure period were statistically significantly decreased in the test groups of 0.172 and 0.444 mg a.s./L. The 36-day mean measured NOEC was 0.027 mg a.s/L based upon the growth of the fish (body length).

[redacted]., 2015b

The chronic toxicity of mefentrifluconazole to zebrafish (*Danio rerio*) was evaluated in a 69-day fish sexual development test under flow-through conditions. Embryos were exposed to a dilution water control and to nominal concentrations of 0.010, 0.21 and 0.041 mg a.s./L, and mean measured concentrations of 0.010, 0.022 and 0.045 mg a.s./L upon which the results are based. During exposure the effect of the test substance on development, survival and growth was evaluated. After sacrifice, fish from all test groups were histologically evaluated for gonad development and sex ratio. Vitellogenin was determined in head and tail of all fish at the end of exposure.

Hatching started simultaneously in all test groups on day 3 and was complete by day 5. Hatching success ranged from 93 - 100% in the control. Survival from hatch to the end of swim-up (day 5) was 100% in the control. No test substance-related effect was observed on the time to start or end of hatching, hatching success and survival from hatch to end of swim-up. In all test groups, larval swim-up started on day 5 of exposure and was complete simultaneously with the control on day 6. No test substance-related effect was observed on the time to swim-up. From the end of swim-up to the end of exposure (day 6 - 68/69) survival was 93 - 100% for the replicates of the control group. The survival from the end of swim-up to the end of exposure as well as the overall survival was not statistically decreased in any treatment group.

There were no signs of toxicity or abnormalities observed among the replicates of the control group and the treatment groups with the exception in the treatment group of 0.021 mg a.s./L of two deformed fish. Due to the low incidence and lack of any observations in the next higher treatment group (0.041 mg a.s./L), these are considered incidental findings and are not test substance related.

In comparison to the control group the mean wet weights and length of the surviving male and female fish at the end of the exposure period were not statistically significantly decreased in the treatment groups.

There was a mean of 50% male fish in the control and means of 50 - 56% males in the treatment groups. There was no statistically significant difference in sex ratio between the control and treatment groups. There were also no significant differences between controls and treatments in maturity index or biomarker vitellogenin concentration in head/tail homogenates. The 69-day NOEC was ≥0.045 mg a.s/L, the highest mean measured concentration tested.

11.6.2 Chronic toxicity to aquatic invertebrates

Four chronic invertebrate aquatic studies were performed, all to CLP and were considered acceptable. Three were to OECD 211 (2012) and one to EPA 850.1350.

Janson G.-M, 2014a

In a 21-day semi-static toxicity test according to OECD 211 (2012), effects of mefentrifluconazole to water fleas (*Daphnia magna*) were examined. Neonates less than 24 hours old were exposed to dilution water and solvent controls and to nominal concentrations of 0.005, 0.010, 0.020, 0.040 and 0.080 mg mefentrifluconazole/L, upon which the results are based, as all analytical measurements were within 80 - 120% of nominal concentrations (81-97% of nominal concentrations). Treatment groups and the controls consisted of 10 replicates containing one daphnid. Assessment of parent mortality and reproduction was conducted daily except for days 3 and 4. Measurements of growth (body weight and length) were made at test termination. After 21 days of exposure, no parent mortality occurred in the control groups and at the test substance concentrations of up to and including the highest concentration tested. Statistically significant differences in the number of offspring per parent were observed at the three highest test substance concentrations. The intrinsic rate of increase and day of first brood were significantly affected at the two highest test substance concentrations. Body length of the parent animals was significantly affected at the highest test substance concentration. The 21-day nominal NOEC was 0.01 mg a.s/L and the 21-day nominal EC_{10} was 0.0175 mg a.s/L, and both were based upon the reproductive endpoint of offspring per female.

Janson G.-M., 2015b

In a 21-day semi-static toxicity test according to OECD 211 (2012), effects of mefentrifluconazole to water fleas (*Daphnia longispina*) were examined. Neonates less than 24 hours old were exposed to a dilution water and solvent control, and to nominal concentrations of 0.015, 0.0225, 0.0338, 0.0507 and 0.0761 mg mefentrifluconazole/L, upon which the results are based, as all analytical measurements were within 80 - 120% of nominal concentrations (95-108% of nominal concentration). All treatment groups and the controls consisted of 10 replicates containing one daphnid. Assessment of parent mortality and reproduction was conducted daily except for days 3 and 4. Measurements of growth (body weight and length) were made at test termination. After 21 days of exposure, no parent mortality occurred in the control groups and at all tested test substance concentrations. Statistically significant differences in the number of offspring per parent were observed at the two highest test substance concentrations and in the intrinsic rate of increase at the highest test substance concentration. Day of first brood, body length, body weight, mobility and age at first reproduction were not affected up to and including the highest test concentration tested. The 21-day nominal EC_{10} was 0.0558 mg a.s/L and the 21-day nominal NOEC was 0.0338mg a.s./L, and both were based upon the reproductive endpoint of offspring per female.

Janson G.-M., 2015a

In a 21-day semi-static toxicity test according to OECD 211 (2012), effects of mefentrifluconazole to water fleas (*Daphnia pulex*) were examined. Neonates less than 24 hours old were exposed to a dilution water and solvent control, and to 0.0125, 0.0188, 0.0282, 0.0423 and 0.0635mg a.s./L which the results are based, as all analytical measurements were within 80 - 120% of nominal concentrations.. All treatment groups and the controls consisted of 10 replicates containing one daphnid. Assessment of parent mortality and reproduction was conducted daily except for days 3 and 4. Measurements of growth (body weight and length) were made at test termination. After 21 days of exposure, no parent mortality occurred in the control groups and at the test substance concentrations

of up to and including the highest concentration tested. Statistically significant differences in the number of offspring per parent and in the intrinsic rate of increase were observed at the two highest test substance concentrations. Day of first brood, body length, body weight, mobility and age at first reproduction were not affected up to and including the highest test concentration tested. The 21-day nominal EC_{10} was 0.0573 mg a.s/L and the 21-day nominal NOEC was 0.0282mg a.s./L, and both were based upon the reproductive endpoint of offspring per female

Dinehart S., 2016 a

The chronic toxicity of mefentrifluconazole to saltwater mysids (*Americamysis bahia*) was evaluated in a 28-day life cycle test under flow-through conditions according to EPA 850.1350. Mysids were exposed to a dilution water control, a solvent control and to nominal concentrations of 1.0, 2.0, 4.0, 8.0 and 16µg a.s./L corresponding to mean measured concentrations of 0.931, 1.50, 3.42, 6.57 and 13.2 µg a.s./L upon which the results were based. Three replicates of 30 mysids were maintained for each test concentration and control. Survival, reproductive success and symptoms of toxicity were assessed throughout the study. Body length and dry weight of the males and females was determined at test termination. No statistically significant and biologically relevant differences were determined between the control and the solvent control data. There was no statistically significant reduction in survival, reproduction, time of first brood release and growth for any test substance treatment. The 28-day mean measured NOEC was ≥0.0132 mg a.s./L.

11.6.3 Chronic toxicity to algae or other aquatic plants

Refer to table 53 and section 11.5.3.

11.6.4 Chronic toxicity to other aquatic organisms

Refer to section 11.5.4.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

Acute aquatic toxicity data on mefentrifluconazole are available for fish, invertebrates, algae and aquatic plants. Fish are the most acutely sensitive trophic group. The lowest reliable acute value is the 96-hour mean measured EC_{50} of 0.532 mg mefentrifluconazole/L for *Oncorhynchus mykiss*, this is > 0.1 mg/L but ≤ 1.0 mg/L and therefore mefentrifluconazole should be classified as Aquatic Acute 1 with an Acute M-factor of 1.

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

Within the classification criteria, mefentrifluconazole is considered ‘not rapidly degradable’.

Mefentrifluconazole has a log K_{OW} value of 3.4, which is lower than the CLP cut-off log K_{OW} value of ≥4. An experimental bioconcentration study in fish is available however, and this gave a growth corrected and lipid normalised kinetic whole fish BCF of 385 for mefentrifluconazole. This is also less than the CLP BCF trigger of 500, therefore, mefentrifluconazole is not considered to have the potential to bioconcentrate.

Chronic/long-term aquatic toxicity data on mefentrifluconazole are available for fish, invertebrates, algae and aquatic plants. Invertebrates are the most chronically sensitive group. The lowest reliable

chronic value is considered to be the 21-day nominal NOEC of 0.01 mg mefentrifluconazole/L for *Daphnia magna*. In the same study however, the 21-day nominal EC_{10} for *D. magna* (based on the same reproduction endpoint) was 0.0175 mg a.s./L. Mefentrifluconazole is 'not rapidly degradable' and based on either of these endpoints it should be classified as Aquatic Chronic 1. The choice of chronic M-factor depends on whether the NOEC or EC_{10} for *D. magna* is chosen as the classification ranges are > 0.001 to $\leq$ 0.01 (M = 10) or > 0.01 to $\leq$ 0.1 (M = 1) and the nominal NOEC is right on this borderline. CLP guidance suggests the EC_{10} appropriate endpoint, and therefore a Chronic M-factor of 1 is proposed.

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Aquatic Acute 1; H400: Very toxic to aquatic life

Acute M-factor = 1

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

Chronic M-factor = 1

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[9] 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.

[9] Single First-Order Rate Model

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 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)
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.

Additional key elements

After the completion of the RAC consultation, RAC became aware of a life-cycle toxicity test on the zebrafish (*Danio rerio*) in a flow through system (140 days exposure of F0 generation; 36 days exposure of F1 generation) conducted in 2017, that was included in the amended draft DAR (2018). The study was conducted according to EPA 850.1500 and EPA 72-5, under GLP and considered acceptable. Based on mean measured concentrations of mefentrifluconazole and on the most sensitive endpoints (effects on female bodyweight and length in the F0 generation, reduction in fecundity (F0) and reduced mean survival day 5-36 in the F1 generation) the overall 36 d F1 & 140d F0 NOEC value was 0.022 mg/L. RAC notes that taking into account this study, the aquatic chronic classification of mefentrifluconazole would not be changed as the value 0.022 mg/L is in same range as EC_{10} for daphnia (0.0175 mg/L).

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 ≤ 0.01 (M=10) or 0.01 < NOEC ≤ 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**.

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

12.1.1 Short summary and overall relevance of the provided information on ozone layer hazard

Due to its low volatility, it is highly unlikely that mefentrifluconazole can deplete the stratospheric ozone layer.

12.1.2 Comparison with the CLP criteria

A substance shall be classified as Hazardous to the Ozone Layer (Category 1) if the available evidence concerning its properties and its predicted or observed environmental fate and behaviour indicate that it may present a danger to the structure and/or the functioning of the stratospheric ozone layer.

The low volatility of mefentrifluconazole precludes an ozone-layer-depleting potential.

12.1.3 Conclusion on classification and labelling for hazardous to the ozone layer

Not classified – conclusive but not sufficient for classification.

13 ADDITIONAL LABELLING

None.

14 REFERENCES

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15 ANNEXES

None