Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at Community level of

2,3,5,6-Tetrafluoro-4-(methoxymethyl)benzyl (Z)-(1R,3R)-3-(2-cyanoprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate; 1R-trans-Z-momfluorothrin

EC Number: Not assigned
CAS Number: 1065124-65-3

CLH-O-0000001412-86-71/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

11 September 2015

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: 1R-trans-Z-momfluorothrin

EC Number: **Not available**

CAS Number: **1065124-65-3**

Index Number: **Not available**

Contact details for dossier submitter: **UK Competent Authority**

Chemicals Regulation Directorate

Health and Safety Executive

United Kingdom

Version number: **1** **Date:** **September 2014**

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***1R-trans-Z-momfluorothrin***
EC number:	***Not available***
CAS number:	***1065124-65-3***
Annex VI Index number:	***Not available***
Degree of purity:	***≥ 83.8 % (typically ≥ 86.0 %)*** ***(Total isomer content ≥ 91.7%)***
Impurities:	***The active substance contains a number of impurities. These have been taken into consideration in the CLH proposal and are not considered to be relevant for the classification and labelling. Further information is provided in the technical dossier.***

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

Current entry in Annex VI, CLP Regulation	Not currently listed
Current proposal for consideration by RAC	Acute Tox 4; H302- Harmful if swallowed STOT-SE 2; H371 – May cause damage to the CNS Aquatic Acute 1: H400 – Very toxic to aquatic life (M = 100) Aquatic Chronic 1: H410 – Very toxic to aquatic life with long lasting effects (M = 100)
Resulting harmonised classification (future entry in Annex VI, CLP	Acute Tox 4; H302- Harmful if swallowed STOT-SE 2; H371 – May cause damage to CNS

Regulation)	Aquatic Acute 1: H400 – Very toxic to aquatic life (M = 100) Aquatic Chronic 1: H410 – Very toxic to aquatic life with long lasting effects (M = 100)

1.3 Proposed harmonised classification and labelling

Table 3: Proposed classification

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.2.	Flammable gases	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.3.	Flammable aerosols	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.4.	Oxidising gases	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.5.	Gases under pressure	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.6.	Flammable liquids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.7.	Flammable solids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.10.	Pyrophoric solids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.13.	Oxidising liquids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.14.	Oxidising solids	Not classified	Not applicable	Not classified	conclusive but not sufficient for

					classification

2.15.	Organic peroxides	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	**Acute Tox 4; H302 – Harmful if swallowed**	Not applicable	-	-
	Acute toxicity - dermal	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
	Acute toxicity - inhalation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	Not classified	Not applicable	Not classified	Data lacking
3.4.	Skin sensitisation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.6.	Carcinogenicity	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.7.	Reproductive toxicity	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	**STOT-SE 2; H371 – May cause damage to the CNS**	Not applicable	-	-
3.9.	Specific target organ toxicity – repeated exposure	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.10.	Aspiration hazard	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment	**Aquatic Acute 1: H400 Very toxic to aquatic**	**Acute M factor = 100**	-	-

		life **Aquatic Chronic 1: H410 Very toxic to aquatic life with long lasting effects**	**Chronic M factor = 100**		
5.1.	Hazardous to the ozone layer	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Pictogram(s): GHS07; GHS08, GHS09

Signal word: Warning

Hazard statements: H302: Harmful if swallowed.

H371: May cause damage to the CNS

H410; Very toxic to aquatic life with long lasting effects

Precautionary statements: Not included in Annex VI of CLP

Proposed notes assigned to an entry:

None

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

1R-trans-Z-momfluorothrin is an active substance in scope of the Biocidal Product Regulation (528/2012). As such, it is subject to the harmonised classification and labelling process in accordance with Article 36 (2) of CLP. There is no current entry on Annex VI of CLP for the substance and the classification and labelling has not been discussed previously within the EU.

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

2.2 Short summary of the scientific justification for the CLH proposal

In a standard acute oral toxicity study, an LD_{50} value of 300 – 2000 mg/kg bw was observed in female rats. This value meets the criteria for classification with Acute Tox 4; H302 - Harmful if swallowed. In the standard acute oral and inhalation studies, and in an acute oral neurotoxicity study, signs of neurotoxicity were observed at doses of 2 mg/l and above. The substance is also a pyrethroid, a class of chemicals known to induce neurotoxic effects (Ray, 1991). Consequently, it is proposed to classify the substance with STOT-SE2; H371 – Causes damage to the central nervous system.

An increased incidence of hepatcocyte adenomas and carcinomas was observed in males rats. However, there is clear evidence suggesting that CAR activation is a plausible mode of action for the formation of these tumours in this species. It has been demonstrated that the substance causes CYP2B induction, hepatocyte hypertrophy and cell replication *in vivo* and *in vitro* for rats by CAR activation. This is consistent with the potential to cause cell foci and tumours in long term studies. In contrast, available data suggest that the substance is capable of inducing CYP2B6 and hypertrophy but then does not increase cell replication in human hepatocytes, which is a prerequisite for tumour formation. Taking into account the supporting *in vivo* and *in vitro* mechanistic studies, it can be argued that 1R-trans-Z-momfluorothrin causes tumours in rats by a mechanism that is not relevant to humans. Therefore, no classification for carcinogenicity is proposed.

From the available aquatic acute toxicity data, fish and invertebrates are the most sensitive trophic group with $L(E)C_{50}$ values in the range 0.001 to 0.01 mg/l. The lowest value is a 96-h LC_{50} of 0.0012 mg/l for fish. The substance should be classified as Aquatic Acute 1 with an M factor of 100.

Chronic toxicity data are available for fish, invertebrates, algae and aquatic plants. The lowest value is a 21-day NOEC for *Daphnia magna* of 0.0005 mg/l. Given this is the range 0.0001 to 0.001 mg/l, The substance should be classified as Aquatic Chronic 1 with an M factor of 100. However, it should be noted the species of fish used for the single chronic test did not reflect the most sensitive fish species (based on the acute data), resulting in a chronic NOEC greater than the LC_{50} values for two fish species. On this basis, it is appropriate to consider the surrogate approach for chronic toxicity to fish. This also results in a classification of Aquatic Chronic 1 with an M factor of 100.

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI of CLP

Not currently listed

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling

The substance is not currently listed in the classification and labelling inventory. The classification proposed by the applicant during the biocides review was as follows

Acute Tox 4; H302 – Harmful if swallowed

STOT SE 1; H370 - Causes damage to organs (central nervous system) if swallowed

STOT RE 2; H373 - May cause damage to organs (Central nervous system, Liver) through prolonged or repeated exposure if swallowed.

Aquatic Acute 1; H400 – Very toxic to aquatic life

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

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

1R-trans-Z-momfluorothrin is an active substance in the scope of Biocidal Products Regulation (528/2012). As such it is subject to the harmonised classification and labelling process in accordance with Article 36 (2) of the CLP.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

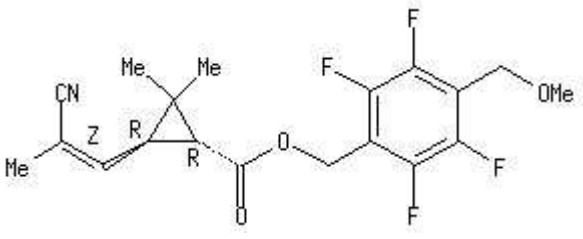
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	Not available
EC name:	Not listed
CAS number (EC inventory):	Not listed
CAS number:	1065124-65-3 [609346-29-4 (All isomers)]
CAS name:	Cyclopropanecarboxylic acid, 3-[(1Z)-2-cyano-1-propen-1-yl]-2,2-dimethyl-, [2,3,5,6-tetrafluoro-4-(methoxymethyl)phenyl]methyl ester, (1R,3R)-
IUPAC name:	2,3,5,6-Tetrafluoro-4-(methoxymethyl)benzyl (Z)-(1R,3R)-3-(2-cyanoprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate
CLP Annex VI Index number:	Not currently listed
Molecular formula:	$C_{19}H_{19}F_4NO_3$
Molecular weight range:	385.35

Structural formula:



RTZ isomer

1.2 Composition of the substance

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
1R-trans-Z-momfluorothrin 2,3,5,6-Tetrafluoro-4-(methoxymethyl)benzyl (Z)-(1R,3R)-3-(2-cyanoprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate	≥ 86.0 %	83.8% - 95.5%	Active substance

The ISO name Momfluorothrin is associated with the IUPAC name 2,3,5,6-Tetrafluoro-4-(methoxymethyl)benzyl (EZ)-(1RS,3RS;1SR,3SR)-3-(2-cyanprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate. In accordance with the guidance on the identification and naming of substances under REACH and CLP, this relates to a mixture of the 8 potential isomers.

However, whilst all isomers are present in the active substance, the major isomer is the RTZ isomer (2,3,5,6-Tetrafluoro-4-(methoxymethyl)benzyl (Z)-(1R,3R)-3-(2-cyanoprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate), which is individually present at > 80%. The other isomers are all individually present at concentrations > 0.1% but < 10%, with the total isomer content in the active substance ≥ 91.7%. As such, the active substance is identified as 1R-trans-Z-momfluorothrin (the RTZ isomer) with CAS number 1065124-65-3 in this CLH proposal.

Full information on the composition of the substance is considered to be confidential and further details are provided in the IUCLID.

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Refer to IUCLID			

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

Table 7: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
None				

1.2.1 Composition of test material

During the review of the active substance under Reg 528/2012, the tested substance was considered to be equivalent to the material outlined above. The human health and environmental data were generated on the substance identified by the manufacturers code S-1563. The minimum purity of S-1563 given in the test reports is 95.2% (based on total isomer content). However, the RTZ isomer is present in S-1563 at a concentration >86%. The purity specified in the human health and environmental sections for S-1563 generally relates to the total momfluorothrin isomer content unless otherwise specified. In some cases, pure isomers (i.e. the RTZ or RTE isomers) were tested. Where this is the case it is specified in the dossier.

1.3 Physico-chemical properties

Table 8: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Powdery white solid	Foster, B. (2012g)	GLP Purity 95.4 %
Melting/freezing point	63.95-73.3 °C (PAI) 73.26-79.07oC (RTZ)	Moseley, R. H. (2011a) Moseley, R. H. (2011b)	EC Method A1 (DSC) OECD 102 GLP Purity 99.9 % (PAI) and 100% (RTZ)
Boiling point	No boiling point observed. Decomposition onset at 326.21 °C (PAI) No boiling point observed. Decomposition onset at 327.48 °C (RTZ)	Moseley, R. H. (2011a) Moseley, R. H. (2011b)	EC Method A2 (DSC) OECD 103 GLP Purity 99.9 % (PAI) and100% (RTZ)
Relative density	1.3432 at 19.7 °C (PAI)	Foster, B. (2012a)	EC Method A3 (gas pycnometer) OECD 109 GLP Purity 99.9 %
Vapour pressure	2.478 x 10^{-7} Pa at 20 °C 1.39 x 10^{-6} Pa at 25 °C (PAI) 4.702 x 10-7 Pa at 20 oC 1.378 x 10-8 Pa at 25 oC (RTZ)	Moseley, R. H.. (2011d) Leslie S (2012)	EC Method A4 (Effusion Method) OECD 104 GLP Purity 99.9 % (PAI) and 99.7% (RTZ)
Surface tension	62.9 mN/m at 19.9 °C (TGAI)	Foster, B. (2011b)	EC Method A5 (Ring Method) OECD 115 GLP Purity 95.4 %
Water solubility	0.933 mg/L at 20 °C (PAI) 0.607 mg/l at 20 oC (RTZ)	Leslie, S. (2010a) Leslie, S. (2010b)	EC Method A6 (column elution) OECD 105 GLP Purity 99.9 % (PAI and RTZ)
Partition coefficient n-octanol/water	LogPow = 2.99 at 25 °C (RTZ isomer) Log Pow = 2.88 at 25oC (RTE isomer)	Wright, D. (2011b) Wright D. (2001c)	EC Method A8 (Shake flask) OECD 107 GLP Purity 99.7 % (RTZ) and 99.7% (RTE)
Flash point	Not applicable as 1R-trans-Z-momfluorthrin is a solid with a melting point > 40 °C		

Flammability	Not highly flammable. The test substance did not ignite. Experience in handling and use indicate that the substance is stable in contact with air and water.	Foster, B. (2012j)	EC Method A10 GLP Purity 95.4 % (TGAI)
Explosive properties	Not explosive. The substance does not contain any groups that are indicative of explosive properties. The oxygen balance is – 184.76% and no exotherms were observed in the DSC.	Foster, B. (2012k)	EC Method A14 GLP Purity 95.4 % (TGAI)
Self-ignition temperature	No relative self ignition observed (up to a temperature of 400 °C)	Foster, B. (2012j)	EC Method A16 GLP Purity 95.4 % (TGAI)
Oxidising properties	Not oxidising. The substance does not contain any groups that are indicative of oxidising properties.	Foster, B. (2011c)	Structural assessment
Granulometry	No data		
Stability in organic solvents and identity of relevant degradation products	No data		
Dissociation constant	No dissociation observed (by spectrophotometric evaluation). (RTZ)_	Wright, D. R. (2011a)	OECD 102 GLP Purity 99.7 % (RTZ)
Viscosity	Not relevant, substance is a solid).		

RTZ = 2,3,5,6-tetrafluoro-4-(methoxymethyl)benzyl (*Z*)-(1*R*,3*R*)-3-(2-cyanoprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate CAS: 1065124-65-3 (*RTZ isomer)*
PAI = 2,3,5,6-tetrafluoro-4-(methoxymethyl)benzyl (EZ)-(1RS,3RS,1RS,3SR)-3-(2-cyanoprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate with RTZ and RTE isomers (in the approximate ratio 9:1)
TGAI = 2,3,5,6-Tetrafluoro-4-(methoxymethyl)benzyl (EZ)-(1RS,3RS;1RS,3SR)-3-(2-cyanoprop-1-enyl)-2,2-Dimethylcyclopropanecarboxylate, isomer content unspecified.

2 MANUFACTURE AND USES

2.1 Manufacture

The substance is manufactured outside of the EU for use as biocidal active substance.

2.2 Identified uses

The substance is used as a biocidal active substance inside the EU.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 9: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
Refer to table 8			

RAC general comment

The active substance tested and reported in experimental toxicity studies is referred to as S-1563. The active substance S-1563 is stated to have a minimum purity of 95.2% w/w (based on the sum of all isomers). The major active isomer of S-1563 is the isomer 1*R*-*trans*-Z-momfluorothrin (sometimes abbreviated as RTZ) with a typical concentration of ≥ 86.0% w/w. Throughout this document, the substance will be referred to as momfluorothrin.

3.1 *Physical Hazards*

3.1.1 Summary and discussion of physical hazards

A standard flammability study (EEC A10) is available and the substance is not classified as a flammable solid. Experience in handling and use indicates it is not pyrophoric and does not react with water to liberate flammable gases. Further, a standard self ignition temperature study (EEC A16) is available and no spontaneous ignition was observed up to a temperature of 400 °C.

The substance does not contain any groups that are indicative of explosive or oxidising properties.

3.1.2 Conclusions on classification and labelling

Not classified, conclusive but not sufficient for classification

RAC evaluation of physical hazards

Summary of the Dossier submitter's proposal

The Dossier Submitter (DS) proposed no classification for physico-chemical properties based on negative results obtained in standard tests. The substance does not contain chemical groups which are indicative of explosive or oxidising properties.

Comments received during public consultation

No comments were received during public consultation (PC).

Assessment and comparison with the classification criteria

Since 1R-trans-Z-momfluorothrin does not have explosive or oxidising properties and is not (auto-)flammable, RAC supports **no-classification** for physico-chemical properties, as proposed by the DS.

HUMAN HEALTH HAZARD ASSESSMENT

The technical material used for the generation of the majority of the human health data is referred to by the manufactures code S-1563 in the study reports. S-1563 is quoted as having a minimum purity of 95.2% (based on total momfluorothrin isomer content) in the study reports, but it is predominantly the RTZ isomer (>86% RTZ). The purity specified in the dossier generally relates to the total isomer content in S-1563 unless otherwise specified. In some cases the pure isomers (i.e. the RTZ or RTE isomers) were tested. Where this is the case it is specified in the dossier.

3.2 Toxicokinetics (absorption, metabolism, distribution and elimination)

3.2.1 Non-human information

The pure RTZ and RTE isomers have also been investigated in the toxicokinetic studies.

S-1563 is rapidly absorbed and extensively metabolised in the rat during single and repeat dose studies by the oral route. The main metabolic pathways include ester cleavage and hydroxylation followed by glucuronidation on available alcohol groups. The parent and metabolites did not accumulate in tissues and are rapidly excreted in urine and faeces (>80%). There are no marked differences across sexes in absorption, distribution, metabolism or excretion.

3.2.2 Human information

Only *in vitro* data with human liver microsomes was available on metabolism of the substance. Exposure of human microsomes resulted in ester cleavage of the parent compound which was consistent with the rat *in vitro* and *in vivo* data.

Dermal absorption of the RTZ:RTE isomers in a ratio of 95:5was investigated in an *in vitro* study with human skin as a 1% solution in ethanol (10g/L). Absorption was low and under current guidance, 6% the RTZ:RTE was available systemically.

No additional data is available on the toxicokinetics in humans.

3.2.3 Summary and discussion on toxicokinetics

1R-trans-Z-momfluorothrin is rapidly absorbed with no bioaccumulation and excreted by the urinary and faecal routes.

3.3 Acute toxicity

The acute toxicity of 1R-trans-Z-momfluorothrin has been investigated in four studies.

Table 10: Summary table of relevant acute toxicity studies

Acute Oral			**Reference**
Method	**LD_{50}**	**Observations and remarks**	
Rat (Sprague Dawley) 5/sex/dose 0, 50, 300, 2000 mg/kg bw in corn oil S-1563 Purity: 95.2% OECD 420 Study compliant with GLP.	> 2000 mg/kg bw males. 300 – 2000 mg/kg bw females.	**2000 mg/kg bw** 2/5 male and 5/5 female rats died. Clinical signs observed at this dose level included urinary incontinence (2 males, 1 female), tremor (1 male, 3 females), salivation (4 males, 4 females), clonic convulsion (4 males, 4 females) , ocular discharge (1 male) and fur staining of the perianal (2 males), perioral (1 male), perinasal areas (2 males) and abdomen (3 males) and tip toe gait (1 female). **300 mg/kg** No deaths in either sex, but tremor (1), urinary incontinence (2) and fur staining of the perianal area (4) in male rats. On necropsy no abnormal findings were seen in the animals that died. In animals that survived to the end of the study, scab and retention of a white substance in urinary bladder of males were observed. These were not considered to be treatment related as they are commonly observed in this strain of rats of the same age and were not dose-dependent.	Deguchi (2010a)
Neurotoxicity (single dose with 14 day observation period) Rat, Sprague Dawley 10/sex/group 0, 30, 80, 200 mg/kg bw in corn oil S-1563 Purity: 95.7% OECD 424 Study compliant with GLP and OECD guidelines	NOAEL:80 mg/kg	**200 mg/kg bw:** One female was found dead on the day after administration. Wetted fur and soiled fur in perianal region of two females at day one and one male at day 2 after the administration respectively. These signs were resolved by day 3. The incidence of tremors in 3 females and salivation in 3 males at 6 hours after administration was increased in detailed clinical observation. Straub tail observed in 1 male and 1 female at 6 hours after administration. There were no abnormal changes in functional tests, body weight, necropsy or histopathological findings. **30 mg/kg bw and 80 mg/kg bw:** No changes were observed in either sex during the 14 day observation period.	Shutoh (2012)
Acute Inhalation			
Method	**LC50**	**Observations and remarks**	
Rat (Sprague Dawley) 5/sex/dose 500, 1000, 2000 mg/m^3 (Actual aerial concentration; 0, 583, 1110, 2030 mg/m^3) MMAD = 5.00, 3.73,	>2000 mg/m^3(2 mg/l)/ 4 hr	Animals in all groups exhibited wet fur and stains around eyes and snout from the end of exposure to 2 hours after exposure. **2000 mg/m^3 (2mg/l)** One female animal died 4 hours after the start of exposure. Tremor of tail (2 females) and tremor (1 female) during exposure. Hypersensitivity (4 males , 1 female), muscular rigidity (1 male), urinary	Deguchi (2011)

4.86 µm S-1563 Purity: 95.7% OECD 403 Study compliant with GLP and OECD guidelines		incontinence (1 female), ataxic gait (1 male, 4 females) and tip toe gait (4 females) after exposure. These signs disappeared within 4 days after exposure. **1000 mg/m^3**(1 mg/l) Ataxic gait in one female, which had disappeared on day 1 after exposure. **500 mg/m^3**(0.5 mg/L) Ataxic gait (1 male), tremor (1 female), muscular rigidity (2 females) and urinary incontinence (1 female). These signs disappeared within two days after exposure. Animals in all groups exhibited wet fur and stains around eyes and snout from the end of exposure to 2 hours after exposure. On necropsy no treatment related findings were reported for any dose group.	
Acute Dermal			
Method	**LD50**	**Observations and remarks**	
Rat Sprague Dawley (5/sex) 2000 mg/kg bw moistened with injection water S-1563 Purity: 95.2% OECD 402 Study compliant with GLP and OECD guidelines	>2000 mg/kg bw	There were no mortalities and no evidence of systemic toxicity at the dose tested.	Deguchi (2010c)

3.3.1 Non-human information

3.3.1.1 Acute toxicity: oral

Oral LD_{50} values of >2000 and 300-2000 mg/kg bw were derived for male and female rats respectively.

3.3.1.2 Acute toxicity: inhalation

In an acute inhalation study, the LC_{50} was measured at greater than >2000 mg/m^3(2 mg/l) for both male and female rats.

3.3.1.3 Acute toxicity: dermal

A dermal LD_{50} value of >2000 mg/kg bw was derived for both male and female rats.

3.3.1.4 Acute toxicity: other routes

No data available

3.3.2 Human information

No data available.

3.3.3 Summary and discussion of acute toxicity

Refer to section 4.2.1

3.3.4 Comparison with criteria

The oral LD_{50} value of 300 – 2000 mg/kg bw for female rats is within the range $300 < LD_{50} \leq 2000$ for classification as Acute Tox 4; H302.

The inhalation LC_{50} was > 2000 mg/m^3 (2 mg/l) with an MMAD of 4.86 µm. Whilst a concentration of 5 mg/L (as included in the classification criteria) was not reached, this was the best atmosphere that could be achieved under controlled experimental conditions. Therefore no classification for acute inhalation toxicity is proposed.

The dermal LD_{50} of >2000 mg/kg bw for rats is above the value for classification provided in the Regulations (i.e. 2000 mg/kg bw). No classification for acute dermal toxicity is proposed.

3.3.5 Conclusions on classification and labelling

Acute Tox 4; H302 Harmful if swallowed

RAC evaluation of acute toxicity

Summary of the Dossier submitter's proposal

The DS proposed to classify 1R-trans-Z-momfluorothrin as Acute Tox. 4 by the oral route (H302). The acute toxicity of R-trans-Z-momfluorothrin was investigated in three GLP studies in rats.

The acute oral study was conducted according to OECD test guideline (TG) 420. Doses tested were 0, 50, 300 and 2000 mg/kg bw. At the highest dose, 7 of 10 rats died (2/5 males and 5/5 females). No deaths were observed at the lower doses. For female rats, an LD_{50} of 300-2000 mg/kg bw was derived, whereas for males the LD_{50} was > 2000 mg/kg bw. The DS proposed to classify the substance as Acute Tox. 4 (H302), based on the female LD_{50} value between 300 and 2000 mg/kg bw.

In an OECD TG 403 acute inhalation toxicity study, rats (5/sex/group) were nose-only exposed to 0, 583, 1110 or 2030 mg/m^3 1R-trans-Z-momfluorothrin for 4 hours. One female rat died at the highest achievable concentration of 2030 mg/m^3 (2.03 mg/L) where particles had an MMAD of 4.86 µm. No classification for acute inhalation is proposed by the DS, as the LC_{50} was > 2000 mg/m^3 (> 2 mg/L) for both males and females.

No mortalities were observed in an acute dermal toxicity study (OECD TG 402) at 2000 mg/kg bw 1R-trans-Z-momfluorothrin . Hence, the DS proposed no classification.

In conclusion, the DS proposed to classify 1R-trans-Z-momfluorothrin as Acute Tox. 4 – H302.

Comments received during public consultation

In their comments, two Member State Competent Authorities (MSCA) and an Industry representative agreed with the proposed classification as Acute Tox. 4. One of the MSCAs suggested to classify 1R-trans-Z-momfluorothrin also as Acute Tox. 4 by the inhalation route

because at the maximum achievable concentration (2 mg/L) one female animal died. Since 50% mortality was not reached at 2 mg/L although it is > 1 and ≤ 5 mg/L, the DS responded that they do not consider this classification proposal appropriate.

Assessment and comparison with the classification criteria

Given that the oral LD_{50} value in female rats fits within the dose limits defining category 4 for acute oral toxicity (> 300 and ≤ 2000 mg/kg bw), RAC supports the conclusion of the DS that 1R-trans-Z-momfluorothrin warrants classification for acute oral toxicity as **Acute Tox 4 (H302)**.

RAC also supports the proposal not to classify 1R-trans-Z-momfluorothrin for acute dermal and inhalation toxicity. For the dermal route, the LD_{50} value in rats is above the threshold value for classification (2000 mg/kg bw). For the inhalation route, the available study does not allow classification, given that the LC_{50} value in both male and female rats is > 2.03 mg/L and higher concentrations could not be tested.

3.4 Specific target organ toxicity – single exposure (STOT SE)

3.4.1 Summary and discussion of Specific target organ toxicity – single exposure

Data from the acute oral, inhalation and neurotoxicity (oral) studies indicates that exposure to 1R-trans-Z-momfluorothrin results in neurotoxicity after a single exposure (refer to table 10).

3.4.2 Comparison with criteria

Classification as either STOT-SE1 or 2 is applicable to substances that have produced non-lethal toxicity in humans, or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant non-lethal toxicity in humans following a single exposure.

Classification as STOT-SE 3 is reserved for transient target organ effects and is limited to substances that have narcotic effects or cause respiratory tract irritation.

Based on the clinical signs seen in the acute inhalation study (see table 11), STOT-SE2 should be considered given that a single exposure via the inhalation route induced signs indicative of acute neurotoxicity at doses of 0.5 mg/l and above, which in some cases persisted for up to 3 days post-exposure.

Table 11: Neurotoxic effects seen following exposure to S-1563 via the inhalation route

	Male						**Female**					
Dose mg/m^3 (mg/l)	**500 (0.5)**		**1000 (1)**		**2000 (2)**		**500 (0.5)**		**1000 (1)**		**2000 (2)**	
	No of animals	Time observed *	No of animals	Time observed *	No of animals	Time observed *	No of animals	Time observed *	No of animals	Time observed *	No of animals	Time observed *
Neurotoxic signs observed during exposure												
Tremor of tail	-	-	-	-	-	-	-	-	-	-	2/5	2h
Tremor	-	-	-	-	-	-	-	-	-	-	1/5	3h
Death	-	-	-	-	-	-	-	-	-	-	1/5	4h
Neurotoxic signs observed post exposure												
Tremor	-	-	-	-	-	-	1/5	1h	-	-	-	-
Hypersensitivity		-	-	-	4/5	1-2h	-	-	-	-	1/4	1h

Ataxic gait	1/5	2 h		-	1/5	2h	-	-	1/5	2h	4/4	2h-d1
Tip toe gait	-	-	-	-	-	-	-	-	-	-	4/4	d2-d3
Muscular rigidity	-	-	-	-	1/5	1h	2/5	2h-1d	-	-	-	-
Urinary incontinence	-	-	-	-	-	-	1/5	2h	-	-	-	-

*Time of observation at which an effect was first and last observed

Animals when exposed to S-1563 via the oral route gave clear signs of neurotoxicity following a single exposure in both the acute oral toxicity study and the acute neurotoxicity study. In the acute oral toxicity study, tremor and urinary incontinence were seen at doses of 300 mg/kg bw. At the next dose level of 2000 mg/kg bw tremor, salivation, and clonic convulsion were seen in both sexes, together with tip toe gait in females. In the acute neurotoxicity study the highest dose tested was 200 mg/kg bw and at this dose, an increased incidence of tremors was seen in female rats (3/9) and increased salivation in males (3/10), together with Straub tail (1/10 males and 1/9) females). Whilst these effects fall within the guidance values for STOT-SE1 via the oral route, this classification is not considered appropriate given the proposed classification of for acute oral toxicity category 4 (section 4.2).

The guidance values for classification for STOT-SE for a dust aerosol are C ≤1 mg/l/4h exposure for category 1 and 5.0 ≥ C >1.0 mg/l/4h for category 2. Based on the neurotoxic effects seen at 2 mg/l (9/10 animals) in the acute inhalation study, classification for STOT-SE2 is proposed. Category 1 could be considered based on the muscular rigidity observed at 0.5 mg/l. However, effects seen at this dose level were only observed in a small number of animals (1 male and 2 females) and are inconsistent with the absence of effects at 1 mg/l.

While the data is not entirely consistent with a clear dose response relationship, 1R-trans-Z-momfluorothrin is a pyrethroid, a class of chemicals known to induce neurotoxic effects (Ray, 1991) so, on balance, classification for STOT-SE2 is proposed. No route of exposure is specified as the clinical findings reported in the acute oral study appear to indicate that inhalation is not the only route that leads to neurotoxicity, although it is noted that the substance is already classified for acute oral toxicity.

The criteria for classification as STOT-SE 3 are not met as the effects are not considered narcotic based on the mode of action of 1R-trans-Z-momfluorothrin which works by blocking sodium channels.

STOT-SE Category 2; H371 May cause damage to the organs (Central Nervous System)

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

Summary of the Dossier submitter's proposal

According to the DS, data from acute oral, inhalation and neurotoxicity (oral) studies indicated that exposure to 1R-trans-Z-momfluorothrin gives rise to neurotoxicity after a single exposure.

In the acute oral toxicity study at the medium dose (300 mg/kg bw), tremors and urinary incontinence were observed in a limited number of animals, while at the highest at the highest dose causing mortality in 70% of the rats (2/5 males and 5/5 females) lethal dose

(2000 mg/kg bw), these clinical signs were more frequently observed and alsoincluded salivation and clonic convulsions in both sexes, and tip toe gait in one female.

In the acute oral neurotoxicity study (10 rats/sex/group), there were no histopathological findings and no changes in functional tests. No clinical signs were observed at the low and mid dose of 30 and 80 mg/kg bw: At the high dose of 200 mg/kg bw, one female died, three females showed tremors and one had a Straub tail. The latter was also observed in one male at 200 mg/kg bw, with three males showing salivation.

In the acute inhalation toxicity study, neurotoxic effects were observed during exposure (only at the highest tested concentration of 2 mg/L, one female died and one to two females showed tremors and tremor of the tail) and post dosing. Clinical signs post-dosing included tremor, hypersensitivity, ataxic and tip toe gait, urinary incontinence and muscular rigidity mostly at 2 mg/L, and lasting up to 3 days. A clear dose response relationship was not observed for muscular rigidity (in 1/5 males at 2 mg/L and in 2/5 females at 0.5 mg/L) or for tremor (only in 1/5 females at 0.5 mg/L).

In the evaluation of the test results and in the weight of evidence analysis for the proposed classification, the DS considered the fact that 1R-trans-Z-momfluorothrin is a pyrethroid, a class of chemicals known to induce neurotoxic effects. The DS further concluded that momfluorothin does not fulfil the criteria for STOT SE 3.

The DS proposed to classify 1R-trans-Z-momfluorothrin as STOT SE 2 – H371 (may cause damage to the nervous system), mainly based on the neurotoxic effects seen at 2 mg/L in the acute inhalation toxicity study, supported by the fact that the substance belongs to the group of pyrethroids, which is known to induce neurotoxic effects.

Comments received during public consultation

Two MSCAs and an industry representative supported the proposed classification in their comments.

Assessment and comparison with the classification criteria

In the available studies, 1R-trans-Z-momfluorothrin induces neurotoxicity following acute oral and inhalation exposure. The lowest doses at which the neurotoxic effects are observed (200 mg/kg bw and 0.5 mg/L for oral and inhalation administration, respectively) in principle fall within the guidance values for STOT SE 1 (≤ 300 mg/kg bw orally, ≤ 1 mg/L via inhalation). However, since there is no clear consistency in effects or dose-response at the next higher dose level (300 mg/kg bw and 1 mg/L, respectively), RAC agrees with the DS that STOT SE 2 (for effective dose levels ranging from 300-2000 mg/kg bw orally and from 1-5 mg/L via inhalation) is more appropriate.

The hazard class STOT SE 3 should cover 'transient' respiratory tract irritation and narcotic effects occurring after single exposure. Although classification in category 3 is primarily based on human data, if available, animal data can be included in the evaluation. Respiratory tract irritation and narcotic effects are generally assessed from standard acute inhalation studies, although it is possible that narcosis could be observed in studies using other routes (see section 3.8 of the CLP Guidance). The acute and sub-acute inhalation studies gave no indication that 1R-trans-Z-momfluorothrin causes an irritant effect in the respiratory tract. As such, RAC concludes that the available data do not indicate that classification for respiratory tract irritation (STOT SE 3) is required.

RAC also agrees with the DS not to specify the route of exposure for STOT SE 2. Indeed, both the oral and inhalation route resulted in clinical signs of neurotoxicity, although for the oral route this is already (partly) covered by the classification for acute toxicity. Hence, the proposal for **STOT SE 2 – H371 (nervous system)** is supported.

3.5 Irritation

3.5.1 Skin irritation

Table 12: Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
Rabbit, New Zealand White 3 males S-1563 Purity: 95.2% OECD 404 Study compliant with GLP	Average individual scores over 24, 48 and 72 hours were: Erythema: 0, 0, 0 Oedema: 0, 0, 0	No classification	Ota, M (2011a)

3.5.1.1 Non-human information

The skin irritation potential of 1R-trans-Z-momfluorothrin has been tested in a standard skin irritation study in three male New Zealand White rabbits. Neither erythema nor oedema was seen in any of the animals.

3.5.1.2 Human information

No data available

3.5.1.3 Summary and discussion of skin irritation

See section 4.4.1.1.

3.5.1.4 Comparison with criteria

1R-trans-Z-momfluorothrin did not cause erythema or oedema in any of the animals tested. Classification as a skin irritant is not required.

3.5.1.5 Conclusions on classification and labelling

Not classified – conclusive but not sufficient for classification

RAC evaluation of skin corrosion/irritation

Summary of the Dossier submitter's proposal

The DS proposed no classification for skin corrosion/irritation. The skin irritation potential of 1R-trans-Z-momfluorothrin was assessed in a standard skin irritation GLP study (OECD TG 404) in three male New Zealand White rabbits. Neither erythema nor oedema was seen in any of the animals; the average individual scores over 24, 48 and 72 hours were equivalent to zero. The DS concluded that 1R-trans-Z-momfluorothrin does not warrant classification for skin corrosion/irritation.

Comments received during public consultation

No specific comments were received during PC, but 2 MSCAs agreed in general terms on the proposed classification for human health hazards.

Assessment and comparison with the classification criteria

In the available study, erythrema was reported in one animal at 1 and 24 hours, but this was not scored since there was no difference in reaction between the application site and the surrounding area. The two other animals did not show signs of erythema or oedema. For classification as a skin irritant in category 2, at least 2 out of 3 animals should demonstrate skin reactions, with a mean score of ≥ 2.3 for erythema and/or oedema. Since this is not the case, RAC supports the proposal for **no classification** for skin corrosion/irritation.

3.5.2 Eye irritation

Table 13: Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
Rabbit, New Zealand White 6 males (3 washed and 3 unwashed) S-1563 Purity: 95.2% OECD 405 Study compliant with GLP and OECD guidelines	Average individual scores over 24, 48 and 72 hours for the unwashed group were: Cornea: 0, 0, 0 Iris: 0, 0, 0 Conjunctiva redness: 0.3, 0.3, 0.3 Conjunctiva chemosis: 0.3, 0.3, 0.3	Not irritating	Ota (2011b)

3.5.2.1 Non-human information

The eye irritation potential of 1R-trans-Z-momfluorothrin has been tested in a standard eye irritation study in male New Zealand White rabbits. No corneal lesions were seen. Iridial congestion (grade 1) was seen in two animals at 1 hour post instillation but had resolved at 24 hours. Conjunctival redness (grade 1) and chemosis (grade 1) was seen in three rabbits at 24 hours, but all ocular reactions had resolved by 48 hours post-application.

3.5.2.2 Human information

No data available.

3.5.2.3 Summary and discussion of eye irritation

1R-trans-Z-momfluorothrin caused mild eye irritation with an average score of 0.3 from 24-72 hours for both conjunctival redness and chemosis. All reactions had resolved themselves by 72 hours post instillation. No classification required.

3.5.2.4 Comparison with criteria

1R-trans-Z-momfluorothrin caused mild, transient irritation of the eye in New Zealand White rabbits. Effects observed between 24 and 72 hours were conjunctival redness and swelling with an average score of 0.3. This does not meet the criteria for classification (average score for iritis ≥ 1, and/or corneal opacity ≥ 1, and/or conjunctival redness ≥ 2, and/or conjunctival oedema ≥ 2, in at least 2 of 3 tested animals).

3.5.2.5 Conclusions on classification and labelling

Not classified – conclusive but not sufficient for classification

RAC evaluation of eye corrosion/irritation

Summary of the Dossier submitter's proposal

The DS proposed no classification for eye corrosion/irritation. The eye irritation potential of 1R-trans-Z-momfluorothrin was tested in a standard eye irritation GLP study (OECD TG 405) in male New Zealand White rabbits. In the three animals where the eyes were not washed after treatment, corneal lesions were not observed, whereas iridial congestion (grade 1) was seen in two animals at 1 hour post instillation, but not thereafter. Conjunctival redness and chemosis, both of grade 1, were observed in all three animals at 24 hours, but these effects had resolved by 48 hours post-application. As the average individual eye irritation scores for both conjunctival redness and chemosis were 0.3 over 24-72 hours, the DS concluded that 1R-trans-Z-momfluorothrin does not warrant classification for eye irritation.

Comments received during public consultation

No specific comments were received during PC, but 2 MSCAs agreed in general terms on the proposed classification for human health hazards.

Assessment and comparison with the classification criteria

Application of 1R-trans-Z-momfluorothrin to the eyes of rabbits resulted in a mild and transient effect on the iris and conjunctivae, whereas the cornea was not affected. Responses seen were completely reversed within 48 hours of application. In all animals the mean scores over 24-72 hours for iritis, conjuctival redness and chemosis were below the threshold values for classification (1, 2 and 2, respectively). RAC therefore agrees that **no classification** for eye corrosion/irritation is required.

3.5.3 Respiratory tract irritation

3.5.3.1 Non-human information

This endpoint was not investigated directly, however the respiratory effects observed during the acute and sub-acute inhalation studies gave no indication that 1R-trans-Z-momfluorothrin causes an irritant effect in the respiratory tract. As such, the available data do not indicate that classification is required for this end-point (see Sections 4.2 and 4.7.1.2).

3.5.3.2 Human information

No data available

3.5.3.3 Summary and discussion of respiratory tract irritation

See section 4.4.3.1.

3.5.3.4 Comparison with criteria

No signs of respiratory tract irritation (as set out in Annex 1: 3.8.2.2.1 of the Guidance on the Application of the CLP Criteria) were observed in the acute or sub-acute inhalation studies that are indicative of respiratory irritation.

3.5.3.5 Conclusions on classification and labelling

Not classified – data lacking

3.6 Corrosivity

3.6.1 Non-human information

1R-trans-Z-momfluorothrin did not lead to full thickness or irreversible skin damage in the skin irritation test nor were any corrosive effects seen in the eye irritation test (see Sections 4.4.1 and 4.4.2). Therefore 1R-trans-Z-momfluorothrin does not meet the criteria for classification as corrosive.

3.6.2 Human information

No data available

3.6.3 Summary and discussion of corrosivity

See section 4.5.1

3.6.4 Comparison with criteria

See section 4.5.1.

3.6.5 Conclusions on classification and labelling

Not classified – conclusive but not sufficient for classification

3.7 Sensitisation

3.7.1 Skin sensitisation

Table 14: Summary table of relevant skin sensitisation studies

Species/Method	Doses	No. sensitised/total no.	Result	Reference
Guinea pig, Hartley (SPF) 20 test, 10 negative	Induction: Intradermal: 5% (w/v) in corn oil.	Test: 0/20 Negative Control (corn	Negative	Ota (2010)

control, OECD 406, Magnusson and Kligman Maximisation test GLP	Topical: 50% in acetone Challenge: 50% in acetone Doses selected from a range finding study. Slight erythema was observed at an intradermal dose of 5%, no skin reactions were observed at a topical dose of 50%. S-1563 Purity: 95.2%	oil): 0/10 Positive control (HCA): 5/5 (100%) HCA control: 0/5.		

3.7.1.1 Non-human information

In a guideline Magnusson and Kligman guinea pig maximisation test, 20 animals were treated with intradermal injections of S-1563 (0.1 ml) at 5% (w/v) in corn oil followed by topical induction with 0.4 ml of 50% S-1563 in acetone. No skin reactions were observed following challenge with 50% S-1563 in acetone. The concurrent positive control with HCA gave the expected results.

3.7.1.2 Human information

No data available.

3.7.1.3 Summary and discussion of skin sensitisation

None of the animals showed a sensitisation response in the guinea pig maximisation test.

3.7.1.4 Comparison with criteria

In a guideline Magnusson and Kligman guinea pig maximisation test, none of the animals tested showed a sensitisation response to 1R-trans-Z-momfluorothrin. A response in 30% of the animals in an adjuvant test is required for classification.

3.7.1.5 Conclusions on classification and labelling

Not classified – conclusive but not sufficient for classification

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal

The potential of 1R-trans-Z-momfluorothrin to cause skin sensitisation was investigated in a GLP Magnusson and Kligman Guinea Pig Maximisation test conducted according to OECD TG 406. The induction phase consisted of intradermal injections of 5% (w/v) of the tested substance in corn oil; topical induction and challenge were performed with 50% 1R-trans-Z-momfluorothrin in acetone. No skin reactions were observed following the challenge.

Based on the absence of skin reactions, the DS proposed no classification for skin sensitisation.

Comments received during public consultation

No specific comments were received during PC, but 2 MSCAs agreed in general terms on the proposed classification for human health hazards.

Assessment and comparison with the classification criteria

RAC supports the conclusion from the DS for **no classification** since none of the animals showed signs of skin sensitisation upon treatment with 1R-trans-Z-momfluorothrin.

3.7.2 Respiratory sensitisation

3.7.2.1 Non-human information

This potential of 1R-trans-Z-momfluorothrin to cause respiratory sensitisation was not investigated directly. However, given that 1R-trans-Z-momfluorothrin does not require classification for skin sensitisation and the sub-acute inhalation study gave no indication of respiratory sensitisation, 1R-trans-Z-momfluorothrin is considered unlikely to be a respiratory sensitiser (see sections 4.6.1 and 4.7.1.2). Therefore no classification is proposed.

3.7.2.2 Human information

No data are available.

3.7.2.3 Summary and discussion of respiratory sensitisation

See section 4.6.2.1.

3.7.2.4 Comparison with criteria

See section 4.6.2.1.

3.7.2.5 Conclusions on classification and labelling

Not classified – Data lacking

RAC evaluation of respiratory sensitisation

Summary of the Dossier submitter's proposal

The potential of 1R-trans-Z-momfluorothrin to cause respiratory sensitisation was not investigated directly. Since the substance does not require classification for skin sensitisation and the sub-acute inhalation study gave no indication of respiratory sensitisation, 1R-trans-Z-momfluorothrin is considered unlikely to be a respiratory sensitiser. Therefore the DS proposed no classification for this hazard class.

Comments received during public consultation

No specific comments were received during PC, but 2 MSCAs agreed in general terms on the proposed classification for human health hazards.

Assessment and comparison with the classification criteria

In the absence of data, RAC has not assessed this hazard class as in other RAC opinions.

3.8 Repeated dose toxicity

Table 15: Summary table of relevant repeated dose toxicity studies

Method	Dose Levels	Observations and Remarks	Reference
Rat studies			
13 week (+ 6 week recovery) oral (dietary) Rat, Wistar 12/sex/group (main study) + 6/sex/group (recovery – control and high dose) OECD 408 Study compliant with GLP and OECD guidelines	0, 300, 1000, 3000, 6000 ppm (0, 23, 76, 223, 485 mg/kg bw/d – males 0, 25, 82, 236, 501 mg/kg bw/d – females) S-1563 Purity: 95.7% Guideline value for classification: ≥100 mg/kg bw/d	**300 ppm (23 mg/kg bw/d (males) and 25 mg/kg bw/d (females))** no test item-related effects were observed in either sex during the treatment and recovery periods. **1000 ppm (76 and 82 mg/kg bw/d)** ↓body weight gains in females (-13%), ↑ absolute and relative liver weights in males (11.2% and 12.1% respectively); brownish pigment in livers (both sexes). ↑ value of phospholipids and cholesterol (males and females), ↑protein content, ↑alpha2-globulin (males). **Doses above the guideline value (>223 mg/kg bw/d)** ↓body weights and ↓body weight gains, ↑absolute liver weight and both sexes. In clinical biochemistry, ↑phospholipids, triglycerides, cholesterol, gamma-glutamyl-transferase, and albumin (both sexes); ↑protein and alpha2-globulin (males) ↑alanine aminotransferase (females). Microscopic examination of livers showed a brownish pigment, hepatocellular hypertrophy (both sexes) and bile duct proliferation In the other organs, brownish pigment in kidneys in both sexes. **Recovery groups** All of the alterations recorded at 1000, 3000 and 6000 ppm, except for the brownish pigment in kidneys, had gradually recovered at the end of the recovery period. NOAEL: 23 mg/kg bw/d LOAEL: 76 mg/kg bw/d*	Sommer (2010)
Neurotoxicity (90 day oral, dietary) Rat, Wistar Male and Female 12/sex/group OECD 424 Study compliant with GLP and OECD guidelines	0, 600, 2000, 6000 ppm (0, 37, 127, 402 mg/kg bw/d (males)) (0, 41, 135, 425 mg/kg bw/d (females)) S-1563 Purity: 95.7% Guideline value for classification: ≥100 mg/kg bw/d	**2000 (127/135 mg/kg bw/d) and 6000 ppm (402/425 mg/kg bw/d):** ↓food consumption (attributed to palatability) in all groups of treated rats during the first week. ↓body weight and body weight gain in both sexes. The overall body weight changes at 2000 ppm and 6000 ppm in males and females were 14.1% and 18.2% or 11.9% and 21.4% of controls respectively. No effect was detected in the FOB Systemic NOAEL: 37 mg/kg bw/d LOAEL: 127 mg/kg bw/d * Neurotoxicity NOAEL: 402 mg/kg bw/d LOAEL: Not determined*	Sommer (2011a)
52 week oral (dietary) Rat, Wistar	0, 200, 500, 1500, 3000 ppm	**200 ppm (11 mg/kg bw/d (males) and 12 mg/kg bw/d (females))** No treatment-related symptoms or mortality, and no	Sommer (2011b)

21/sex/group OECD 452 Study compliant with GLP and OECD guidelines	(0, 11, 27, 83, 169 mg/kg bw/day - males 0, 12, 34, 103, 199 mg/kg bw/day – females) S-1563 Purity: 95.7% Guideline value for classification is: 25 mg/kg bw/d	effects in the FOB, ophthalmoscopy and urinalysis. **500 ppm (27 mg/kg bw/d (males) and 34 mg/kg bw/d (females))** ↓Body weight (-12.1%) and ↓body weight gain (-4.7%) in females. ↑Absolute (13.2% in males) and relative liver weights (17.9% females). Histologically, brown pigment deposition was seen in the liver with a dose-associated distribution. Slight ↑ in levels of phospholipids in females and mainly centrilobular hepatocellular hypertrophy in males and females were observed at minimal severities and incidences. ↑incidence of brown pigment deposition in the kidneys (females). **Doses above the cut-off for classification** A gradual worsening of treatment-related effects seen at 500 ppm was observed. Including ↓Body weight and ↓body weight gains, ↑increase total cholesterol, phospholipids, protein and albumin content, GGT and ↑total bilirubin values (both sexes); ↑ alpha1-globulin and alpha2-globulin, triglycerides (males); A/G ratio (females). ↑absolute and relative liver weights ↑brown pigment deposition in the liver. ↑incidence of mainly centrilobular hepatocellular hypertrophy (both sexes). ↑ incidence in bile duct proliferation (female) ↑incidence of brown pigment deposition in the kidney. Diffuse follicular cell hypertrophy in the thyroid of 1 female and 7 males. ↓food consumption was also observed in females of the highest dose group. In haematology alterations including shortening of PrT and PT); ↑increased incidence of diffuse acinar hypertrophy in the mandibular salivary gland (10 males, 16 females) NOAEL: 12 mg/kg bw/d LOAEL: 34 mg/kg bw/d*	
Dog studies			
14 day dietary study in Beagle dogs Non-guideline Male and female 1/sex/group Range finding study conducted in advance of the 13- and 52-week studies. Detail has been provided for completeness and only longer term studies have been used for classification	0, 50, 500, 1000 mg/kg bw/d Daily dose S-1563 Purity: 99.1%	**50 mg/kg bw/d**- vomiting and watery faeces on one single occasion. **500 mg/kg bw/d**- vomiting and watery faeces in both sexes, predominantly over the first 5 days **1000 mg/kg bw/d**- vomiting, tremors, salivation and watery faeces in both sexes, predominantly over the first 4 days. Male killed in extremis due to persistent signs and additional tachypnea. No treatment related changes in clinical chemistry, haematology, body weight, food consumption, organ weights or changes at necropsy at any dose level. NOAEL: 50 mg/kg bw/d LOAEL: 500 mg/kg bw/d*	Braun, L (2008)

purposes. S-1563 Purity: 95.7%			
OECD 409 13 week dietary dog study (+ 6 week recovery) Male and female Main study- 4/sex/group Recovery group- 2/sex/group	0, 50, 200, 600 mg/kg bw/d Daily dose S-1563 Purity: 95.7% A guideline value of 100 mg/kg/d is considered for classification based on the value defined for the rat 90 day study.	**50 mg/kg bw/d**- no significant adverse effects. **Doses above the cut-off for classification** Vomiting, watery faeces and changes in biochemical parameters (e.g. increased cholesterol and triglycerides and decreased ALT and alpha-1-globulin) were reported at doses above the guideline value (200 and 600 mg/kg bw/d). In addition, absolute and relative liver weight increases and hepatocellular hypertrophy were recorded in both sexes at 600 mg/kg bw/d. These findings are consistent with those reported in the rat and of no concern for classification. NOAEL: 200 mg/kg bw/d LOAEL: 600 mg/kg bw/d*	Braun, L (2011)
OECD 452 52 week dietary dog study Male and female Main study- 4/sex/group	0, 25, 100, 400 mg/kg bw/d Daily dose S-1563 Purity: 95.7% A guideline value of 25 mg/kg/d is considered for classification based on the value defined for the rat (90 day study plus application of the Haber rule).	**25 mg/kg bw/d**: The only effect was decreased alpha-1-globulin at week 8 in females (0.7-fold) **At doses above the guideline value for classification** Liver hypertrophy was noted at 100 mg/kg bw/d (2/4 males and 1/4 females) and 400 mg/kg bw/d (4/4 males and females). Clinical signs included vomiting, watery faeces and salivation. The clinical biochemistry analysis showed a trend towards increased levels of triglycerides in both sexes with statistical significance generally observed at weeks 4, 8 and 13. Overall, there was no toxicity of concern at dose levels applicable to classification. NOAEL: 25 mg/kg bw/d LOAEL: 100 mg/kg bw/d*	Braun, L (2012)

*values cited in the CAR

3.8.1 Non-human information

3.8.1.1 Repeated dose toxicity: oral

Rat

There are two studies investigating the repeat dose toxicity in the rat via the oral route; a 13 week (+ 6 week recovery) study and a 52 week study.

In a 13 week study to investigate the sub-chronic toxicity of 1R-trans-Z-momfluorothrin, rats were dosed at concentrations of 300, 1000, 3000 and 6000 ppm S-1563 in their diets for 13 weeks

(equivalent to 23, 76, 223, 485 mg/kg bw/d (males) and 25, 82, 236, 501 mg/kg bw/d (females)). This was followed by a 6-week recovery period for the control (0 ppm) and highest dose groups.

At doses below the guideline value of 100 mg/kg bw/d, the main effects were on body weight and the liver. Body weight was reduced in females by 12.1% while the absolute and relative liver weight increased in males by 11.2% and 12.1% respectively. Microscopic examination of livers, found a brownish pigment in the livers of males (4/12) and females (10/12). Changes in clinical biochemistry included increased levels of phospholipids and cholesterol in males and females, increases in protein and alpha2-globulin in males. These alterations in the clinical biochemistry are considered to be indicative of functional changes in the liver.

At doses above the guideline these effects gradually worsened with increasing dose. The liver was macroscopically enlarged in males. Hepatocellular hypertrophy and bile duct proliferation were recorded and ultrastructural changes showed a moderate amplification and enlargement of the smooth endoplasmic reticulum (SER), an augmentation of rough endoplasmic reticulum (RER), and an intracellular accumulation of solid dark bodies (lysosomes). Changes in clinical biochemistry included increased levels of phospholipids, triglycerides, cholesterol, gamma-glutamyl-transferase, and albumin in males and females, increases in protein and alpha2-globulin in males and in alanine aminotransferase in females. These alterations in the clinical biochemistry are considered to be indicative of functional changes in the liver. Brownish pigment in the kidneys was seen in both sexes together with a change in the urinalyses in females at the highest dose. A slight diffuse acinar hypertrophy in mandibular glands was also seen in both sexes and is considered treatment related. Minor changes to some haematology parameters were reported at the higher doses, including increased reticulocytes count and reduced mean corpuscular volume and mean corpuscular volume in both sexes and reduced haemoglobin and haematocrit in females.

Following the 6 week recovery period, all of the reported changes, except the brownish pigment in the kidneys, had reversed.

In the 52 week study in rats, groups of 21 male and 21 female rats received S-1563 in their diet at concentrations of 0, 200, 500, 1500, 3000 ppm (equivalent to 0, 11, 27, 83, 169 mg/kg bw/d (males) and 0, 12, 34, 103, 199 mg/kg bw/d (females)). At doses marginally higher than the guideline for classification (25 mg/kg bw/d) based on the 13 week study and the application of Haber's rule the main effects were on the liver and body weight. At doses 27 mg/kg bw/d mean absolute liver weights were increased in males at by 13.2% and in females the mean relative liver weight increased by 17.9%. These changes were accompanied by slight increases in phospholipid levels in females and mainly centrilobular hepatocellular hypertrophy at minimal severities and incidences in both sexes. In females a decrease in body weight of 15% as compared to the controls was recorded. At doses in excess of the cut-off (>82.6 mg/kg bw/d), an increased deposition of a brown pigment was reported in liver and kidney along with increased incidence of centrilobular hypertrophy. As with the 13 week study, the incidence of these effects increased at higher doses. At doses above the cut off, liver enlargement was found to be associated with hepatocyte hypertrophy, bile duct proliferation and lipofuscin pigment deposition. In addition diffuse follicular cell hypertrophy was seen in the thyroid and slight diffuse acinar hypertrophy of the mandibular gland was at the highest dose. Changes in clinical chemistry and haematology were observed at doses of 83 mg/kg bw/d and above. These findings are consistent with the results reported in the 13 week study in rats.

Dog

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1R-TRANS-Z-MOMFLUOROTHRIN

There are two dietary studies available for classification purposes: one 13 week study with a recovery period and a 52 week study.

Using the guideline value (100 mg/kg bw/d s-1563) as defined for the rat in a 13 week study, there were no treatment related findings at 50 mg/kg bw/d S-1563 in the dog. At doses greater than the cut-off value (200 and 600 mg/kg bw/d), there were reports of vomiting, watery faeces and changes in biochemical parameters e.g. increased cholesterol and triglycerides and decreased ALT and alpha-1-globulin. These findings suggested that the liver was a potential target organ and were consistent with the results in the rat.

A 52 week study in the Beagle dog was submitted in accordance with OECD guideline 452 and to GLP standards (Braun, 2012). Using the guideline value (25 mg/kg bw/d) as defined for the rat in a 52 week study, the only effect at 25 mg/kg bw/d in the dog was a decrease in alpha-1-globulin (0.7-fold). This effect was present at week 8 in females only. Due to the transient nature of the decrease in female dogs and the inconsistency across sexes, this finding is not a concern for classification purposes. At higher doses (100 and 400 mg/kg bw/d), there were reports of vomiting, watery faeces, salivation and changes in biochemical parameters e.g. increased cholesterol and triglycerides and decreased ALT and alpha-1-globulin. As these findings are consistent with those reported in the rat, they are not a concern for classification.

3.8.1.2 Repeated dose toxicity: inhalation

Table 16: Summary table of relevant repeated dose toxicity studies

Method	Dose Levels	Observations and Remarks	Reference
28 day inhalation (nose only) Rat (Sprague Dawley) 10/sex/group OECD412 Study compliant with GLP and OECD guidelines	Nominal concentration: 0, 50, 150, 300 mg/m^3 (Analytical concentration: 0, 62.2, 170, 320 mg/m^3) MMAD: 4.49, 5.00, 5,07µm respectively Purity: 95.7% Guidance value for classification ≤0.6 mg/l	All doses tested were below the cut-off value for classification. Following exposure, signs of transient effects on the CNS and an adaptive response in liver were seen. None of these effects (which are summarized below) were considered sufficient to justify classification. Clinical signs included transient tremor, ataxic gait, muscular rigidity and hypersensitivity after exposure in one male in the 300 mg/m^3 group and tip toe gait in 1 female each of the 150 and 300 mg/m^3 groups. Increased liver weight of >10% was observed in males of all test groups and females of the 150 and 300 mg/m^3 test groups only reaching statistical significance in females of the top dose group. Increases in relative liver weight were statistically significant in all dose groups in males and the top two dose groups in females At the highest dose, males showed an increase in ASAT and changes in blood chemistry (increased total cholesterol and decreased blood glucose level). Slight increases in total cholesterol were found in males of the 150 mg/m^3 group. In females of the 150 and 300 mg/m^{3}groups, increased liver weight was accompanied by an increase in total cholesterol and phospholipids. Changes in the brain weight of males and the adrenal weight of females in the 300 mg/m^3 group NOAEL: 50 mg/m^3 LOAEL: 150 mg/m^{3*}	Deguchi, Y. (2011)

*values proposed in the CAR

Rat

Clinical signs observed were tremor, ataxic gait, muscular rigidity and hypersensitivity after exposure in 1 male in the 300 mg/m^3 group and tip toe gait in 1 female each of the 150 and 300 mg/m^3 groups. These disappeared after exposure on the same day or before exposure on the next day and, with the exception of hypersensitivity which was seen on Day28, were not observed after Day 3 of exposure. Wet fur was seen in all groups, however was seen to persist longer in the animals in the two highest dose groups suggesting general conditions were worsened by exposure to the test substance, with the result of extending the time to disappearance of wet fur. Thus, wet fur is possibly a secondary effect of the exposure to the test substance.

Increased liver weight was observed in both males and females of the 150 and 300 mg/m^3 test groups. At the highest dose, males showed an increase in aspartate aminotransferase and changes in blood chemistry (increased total cholesterol and decreased blood glucose level). Only slight increases in total cholesterol were found in males of the 150 mg/m^3 group; these were not statistically different from the controls and fell within the range of the historical data. No

underlying histopathological changes were observed in this group and so the observed change is considered to be of no toxicological significance. In females of both the 150 and 300 mg/m^3 dose groups, increased liver weight was accompanied by an increase in total cholesterol and phospholipids. These results suggest a possible effect of 1R-trans-Z-momfluorothrin on lipid and glucose metabolism in the liver. Changes seen in the brain weight of males and the adrenal weight of females in the 300 mg/m^3 group were considered to be of no toxicological significance because there was no underlying effect observed in related haematology, blood chemistry, or histopathological examination.

3.8.1.3 Repeated dose toxicity: dermal

Table 17: Summary table of relevant repeated dose toxicity studies

Method	Dose Levels	Observations and Remarks	Reference
28 day dermal Rat/ (SD) 10/sex/group OECD 410 Study compliant with GLP and OECD guidelines	0, 100, 300, 1000 mg/kg bw/d 6 hours/day	No treatment related effects at any dose	Ogata, H. (2012)

3.8.1.4 Repeated dose toxicity: other routes

No data available.

3.8.1.5 Human information

No data available.

3.8.1.6 Other relevant information

Carcinogenicity studies in rats and mice are presented in section 4.10. All the doses tested were above the 25 mg/kg bw/d and so according to the criteria effects observed at this level are not relevant for classification.

3.8.1.7 Summary and discussion of repeated dose toxicity

The short term repeat dose toxicity of 1R-trans-Z-momfluorothrin was investigated in rats and dogs. The long term repeat dose toxicity was addressed in the carcinogenicity studies with rats and mice (section 4.10) and has been reported as appropriate.

Body weight

At doses below the respective guideline values for classification, the effects of 1R-trans-Z-momfluorothrin are primarily on liver and body weight in rats. A decrease in body weight was reported in female rats at 13 weeks but this was not seen in males or in studies of longer duration. This effect worsened with increasing dose and was also reported in males at higher dose levels.

In dogs and mice, no effects were noted at doses below the guidance values for classification.

Liver

Effects on the liver were observed following treatment via both the oral and inhalation routes. Increased liver weight was reported in males and females at doses below or marginally above the guidance values for the 28 day inhalation and 13 and 52 week oral studies in rats. This was accompanied by changes in clinical biochemistry, hypertrophy and the deposition of a brown pigment in the liver.

In dogs and mice, no effects were noted at doses below the guidance values for classification.

Kidney

In the 52 week study, females dosed with 34mg/kg bw/d (above the guidance value) had deposition of brown pigment in the kidney. No further effects were seen in the kidney.

In dogs and mice, no effects were noted at doses below the guidance values for classification.

3.9 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

3.9.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

The repeated-dose toxicity of 1R-trans-Z-momfluorothrin has been investigated via the oral, dermal and inhalation routes in rats, mice and dogs. As discussed in Section 4.1.7, the consistent finding was an effect on the liver following dosing via the oral and inhalation exposure. The liver effects included increases in liver weight, liver hypertrophy and alterations in liver function as indicated by clinical biochemistry changes.

3.9.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

Classification as either STOT-RE1 or 2 is applicable to substances that have produced significant toxicity in humans, or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure.

Guidance values for classification for STOT-RE Category 2 are set at <10 C $\leq$ 100 mg/kg bw/d based on a 90 day study or $\leq$300 mg/kg bw/d for a 28 day study in rats. In rats, hepatocyte hypertrophy and alterations in the liver function as shown by increased triglycerides, phospholipids and aspartate aminotransferase are consistently seen in the repeated-dose studies. However, while these changes occur at levels that would merit classification as STOT-RE they are considered to be adaptive and do not produce a significant adverse toxicological effect and so classification for STOT-RE is not justified.

In dogs and mice all treatment related responses were seen at levels above the guidance values for classification.

3.9.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

1R-trans-Z-momfluorothrin does not require classification for specific target organ toxicity following repeated dose.

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 DS proposed no classification for STOT RE for 1R-trans-Z-momfluorothrin based on the analysis of several standard repeated dose toxicity studies in rats, up to 52 weeks duration with oral exposure and with 28-day dermal or inhalation exposure, all GLP and OECD TG compliant. In addition, three oral studies in dogs (a range-finding study and two sub-chronic OECD TG compliant studies, of 13- and 52-weeks duration) as well as two GLP and OECD TG compliant long-term oral studies (a 104-week study in rats and a 78-week study in mice) were summarised and assessed by the DS.

No effects were seen in the 28-day dermal study in rats, with a NOAEL at the highest tested dose of 1000 mg 1R-trans-Z-momfluorothrin/kg bw/day. In the 28-day inhalation study in rats, the liver was the target organ. Although all concentrations tested (up to 320 mg/m^3) were below the guidance value for classification, the DS considered the liver effects induced by 1R-trans-Z-momfluorothrin (changes in liver weight and on some biochemistry parameters related to liver function, but not on liver histopathology) to be adaptive in nature and therefore not sufficient to justify classification.

Liver was also the target organ in the long-term oral study in mice, with 1R-trans-Z-momfluorothrin treatment resulting in increased liver weight accompanied by hepatocellular hypertrophy and, at the highest dose, enhanced single cell necrosis and brownish liver pigmentation. However, according to the DS, all effective dose levels in this study were above the guidance value for classification. The same was true for dogs, where liver toxicity (indicated by (reversible) biochemical changes and increases in liver weight and hepatocellular hypertrophy) was only observed at doses above the respective guidance values for oral 13- and 52-week studies.

In rats, the main findings in an oral 13-week (+ 6-week recovery) and 52-week study were on body weight and the liver. At doses at or below the respective guidance values (100 and 25 mg/kg bw/day, respectively) the effects were relatively small, with up to 12.5% decrease in body weight gain (females only), increases in liver weight in males (11.3-13.3% in the 13- and 52-week study) and females (17.9% in the 52-week study), changes in clinical biochemistry indicative of functional liver changes and some brown pigmentation (lipofuscin) in the liver. At doses above the respective guidance values, these effects increased in incidence and/or severity with increasing dose, and were associated with histopathological changes characterised by hepatocellular hypertrophy, bile duct proliferation and ultrastructural modifications. Similar non-neoplastic liver findings were observed in the oral long-term study in rats, but only at doses above the guidance value for classification. In addition, from the 13-week study with a 6-week recovery period it appeared that most effects had reversed.

Other organs were also affected in the 13-, 52- and 104-week oral rat studies, but only at doses above the respective guidance values for classification. These included the kidney (deposition of a brown pigment similar to that in liver, without further evidence of kidney

injury), mandibular glands (diffuse acinar hypertrophy) and, in the 52- and 104-week studies, the thyroid (follicular cell hypertrophy). The thyroid finding was considered secondary to the liver effects.

Overall, only the liver findings in rats occurred at dose levels relevant for classification. However, as the effects at these levels only related to increased weight, biochemical alterations and lipofuscin deposition without hepatocellular hypertrophy and bile duct proliferation of high severity/incidence, and were reversible, the DS concluded that these changes are considered to be adaptive and do not constitute a significant adverse toxicological effect warranting classification according to CLP criteria. Therefore, the DS proposed no classification for STOT RE for 1R-trans-Z-momfluorothrin.

Comments received during public consultation

No specific comments were received during PC, but 2 MSCAs agreed in general terms on the proposed classification for human health hazards.

Assessment and comparison with the classification criteria

The repeated dose toxicity of 1R-trans-Z-momfluorothrin has been investigated via the oral, dermal and inhalation routes, in three species (rat, mouse and dog). Special studies investigating possible immunotoxic (28-day study; Hosako, 2011) or neurotoxic (90-day study; Sommer, 2011a) effects of 1R-trans-Z-momfluorothrin following oral administration to rats were also provided in the CLH report.

Oral

In rats, the repeated dose toxicity was investigated in a 13-week (+ 6-week recovery), 52-week and 104-week study.

In the 13-week study, 1R-trans-Z-momfluorothrin was administered in the diet at doses of 0, 300, 1000, 3000 or 6000 ppm (23, 76, 223 or 485 mg/kg bw/day for males and 0, 25, 82, 236 or 501 mg/kg bw/day for females). The control and high dose groups subsequently received a control diet during a 6-week recovery period. At doses below the guidance value of 100 mg/kg bw/day, body weight gain was reduced by 12.5% in females, whereas in males the absolute and relative liver weights were increased by 11.3% and 12.1%, respectively. Upon microscopic examination a brownish pigment of minimal severity was observed in the livers of males (4/12) and females (10/12). Functional changes in the liver were demonstrated by changes in clinical biochemistry (increased levels of phospholipids and cholesterol in both sexes and increases in alpha2-globulin in males).

In the 13-week study, doses above the guidance value for classification as STOT RE 2 (≤ 100 mg/kg bw/day) showed a gradual increase in incidence and/or severity of the above-mentioned effects with increasing dose. In addition, from 3000 ppm liver enlargement was seen in male rats, and hepatocellular hypertrophy (mainly diffuse) and bile duct proliferation (both of minimal severity) in both sexes. Ultrastructural assessment of the liver samples from the 6000 ppm group revealed a moderate amplification and enlargement of the smooth endoplasmic reticulum (SER), an augmentation of rough endoplasmic reticulum (RER), and an intracellular accumulation of solid dark bodies (lysosomes). Other findings in both sexes included deposition of a brownish pigment in the kidneys and slight diffuse acinar hypertrophy in the mandibular glands. Upon recovery, the effects had considerably diminished or were no longer seen.

In the 52-week study, rats received 1R-trans-Z-momfluorothrin in their diet at 0, 200, 500, 1500 or 3000 ppm (0, 11, 27, 83 or 169 mg/kg bw/day (males), and 0, 12, 34, 103 or 199 mg/kg bw/day (females)). Results were comparable to the 13-week study: at doses around the (extrapolated) guidance value of 25 mg/kg bw/day for classification as STOT RE 2, the main effects were on the liver and body weight. Around the guidance value of 25 mg/kg

bw/day, body weight was reduced by 12.1% in females and liver weights were increased in both males (absolute, 13.3%) and females (relative, 17.9%), and histopathologically a brownish pigmentation was seen in the liver (in 1/21 males and 2/21 females). In females, also a slight increase in phospholipid levels was observed, and an increased incidence of hepatocellular hypertrophy (mainly centrilobular, reported to be of minimal severity). At higher doses these effects increased in incidence and/or severity, and included also an increased incidence of bile duct proliferation (in females at 3000 ppm only). Further effects included deposition of a brown pigment in the kidney, and at 3000 ppm also increased incidences of diffuse acinar hypertrophy in the mandibular glands and thyroid follicular hypertrophy.

In the 104-week study (see also the section on Carcinogenicity), non-neoplastic liver findings started from 1500 ppm (73 mg/kg bw/day), i.e. at dose levels above the (extrapolated) guidance value of 12.5 mg/kg bw/day. At these dose levels also brown pigmentation in the kidney, diffuse acinar hypertrophy in the mandibular glands and thyroid follicular hypertrophy were seen, similar to the rat studies of shorter duration.

Liver was also the target organ in a long-term oral study in mice (see also the section on Carcinogenicity), with 1R-trans-Z-momfluorothrin treatment resulting in increased liver weight (from 600 ppm (72 mg/kg bw/day) in males and from 2500 ppm (427 mg/kg bw/day) in females), enhanced hepatocellular hypertrophy (from 2500 ppm) and, at the highest dose of 5500 ppm, enhanced single cell necrosis and brownish liver pigmentation. However, all effective dose levels in this study were above the (extrapolated) guidance value for classification (16.7 mg/kg bw/day for a 78-week study).

In dogs, the repeated dose toxicity was investigated in a 13-week (+ 6-week recovery) and 52-week study, following a 14-day range-finding study (all with administration of 1R-trans-Z-momfluorothrin via gelatin capsules).

In the 13-week study, with doses of 0, 50, 200 or 600 mg/kg bw/day, there were no treatment-related findings at the low dose. At doses above the guidance value for classification (100 mg/kg bw/day), some minor findings were observed at the mid dose whereas at the high dose there were clinical signs (vomiting, watery faeces and excess salivation) and findings indicative of an effect on the liver ((reversible) changes in biochemical parameters such as increases in cholesterol and triglycerides and decreases in glucose, alanine aminotransferase and alpha-1-globulin, increased liver weights and centrilobular hepatocellular hypertrophy).

In the 52-week study, with doses of 0, 25, 100 or 400 mg/kg bw/day, no adverse effects were observed at the level of the (extrapolated) guidance value for classification (25 mg/kg bw/day). Higher doses resulted in clinical signs (vomiting, watery faeces, salivation), changes in biochemical parameters and hepatocellular hypertrophy, but not in increased liver weight.

Inhalation

In a 28-day study, rats were exposed nose-only for 4 hours per day with 0, 62.2, 170 or 320 mg/m^3 1R-trans-Z-momfluorothrin. All doses tested are below the (extrapolated) guidance value for classification (900 mg/m^3 for a 28-day study with 4 hour exposure per day). The main findings in this study included clinical signs of neurotoxicity and effects on liver weight and on some biochemistry parameters. The clinical signs however only occurred in a limited number of animals (tremor, ataxic gait and muscular rigidity were observed in one male of the high dose group and tip toe gait in one female each of the mid and high dose groups) and were transient in nature (disappearance within one day, and occurrence only during the first three days of exposure). Increased liver weights were observed in both males and females of the mid and high dose group. In males this was accompanied by increases in aspartate aminotransferase and total cholesterol and a

decrease in blood glucose level at the high dose, in females with increases in total cholesterol and phospholipids at both doses. In both sexes there were no correlating histopathological findings.

Dermal
In a 28-day dermal study, rats were treated with 0, 100, 300 or 1000 mg 1R-trans-Z-momfluorothrin/kg bw/day. No treatment related effects were seen at any dose.

Conclusion
From the inhalation study in rats and the oral studies in rats, mice and dogs it can be concluded that liver is the target organ of 1R-trans-Z-momfluorothrin toxicity. In mice and dogs, effects on the liver were only observed at dose levels above the guidance values for classification. In rats they also occurred at levels that would warrant classification. However, histopathological findings occurred in a few animals and were transient and of minimal severity. RAC agrees with the DS that this does not represent significant adverse toxicity. Besides, the liver effects were shown to be reversible to a large extent. Furthermore, 1R-trans-Z-momfluorothrin was shown not to be immunotoxic or neurotoxic upon repeated exposure. Therefore, RAC supports the **no classification** proposal for STOT RE for 1R-trans-Z-momfluorothrin.

3.10 Germ cell mutagenicity (Mutagenicity)

Table 18: Summary table of relevant in vitro and in vivo mutagenicity studies

In vitro data				**Reference**
Method	**Organism/strain**	**Concentrations tested**	**Result**	
Bacterial reverse mutation test OECD 471 Study compliant with GLP and OECD guidelines	*S.typhimurium* TA1535, TA1537, TA98, TA100 and *E.coli* WP2uvrA	156 – 5000 µg/plate	Negative both in the presence and absence of S9.	Kitamoto, (2009a)
In vitro chromosome aberration assay in mammalian cells OECD 473 Study compliant with GLP and OECD guidelines	Chinese hamster lung cells (CHL/IU)	Experiment 1 6 hours treatment/18 hours recovery + and –S9 -S9- 39.1, 78.1, 156, 313, 625 and 1250 µg/mL +S9- 40.0, 60.0, 80.0, 100, 120, 140 and 160 µg/mL Experiment 2 24 treatment –S9; 6 hours treatment/18 hours recovery +S9 -S9- 4.88, 9.77, 19.5, 39.1, 78.1 and 156 µg/mL +S9- 40.0, 60.0, 80.0, 100, 120, 140 and 160 µg/mL	Experiment 1- there was no increase in the incidence of structurally or numerically aberrant cells in the absence of S9 but a marginal increase in the incidence of structurally aberrant cells was noted in the presence of S9 at 120 and 140µg/ml. Experiment 2- No increase in structural or numerical aberrations in the absence of S9 at any dose level. The marginal increase in structural aberrations was reproduced in the presence of S9 at 100, 120 and 140µg/ml. Cytotoxicity was seen both in the presence and absence of S9 mix (24 hours treatment). A dose-dependent growth inhibition was observed in the absence of S9 mix (6 hours treatment and 18 hours recovery), but there was no marked inhibition to growth rate of 50% or lower. This result is considered positive given the marginal increase in the incidence of structurally aberrant cells in the presence of S9.	Kitamoto (2009b)
In vitro gene mutation assay OECD 476 Study compliant with GLP and OECD	Chinese Hamster V79 cells	Experiment 1 -S9- 7.7, 15.3, 30.5, 61.0, 91.5 and 122.0 µg/mL +S9- 15.3, 30.5, 61.0, 76.8, 91.5,	In the absence of metabolic activation, no relevant and reproducible increase was observed in the mutation frequency. The induction factor exceeded the threshold of 3x the solvent control in experiment 1 at 15.3 µg/ml, but this was not repeated in the parallel culture. All values were within the historical control ranges.	Wollny (2011)

guidelines		106.8 and 122.0 µg/mL Experiment 2 -S9- 7.7, 15.3, 30.5, 61.0, 91.5 and 122.0 µg/mL +S9-7.7, 15.3, 30.5, 61.0, 76.3 and 91.5 µg/mL	In the presence of metabolic activation, mutation frequencies were less than 3x the vehicle control for all treated groups. The result is negative in both the presence and absence of S9 with no dose related increases in mutation frequency in any experiment. All mutation rates were within the relevant historical control ranges.	
***In vivo* data**				**Reference**
Method	**Organism/strain**	**Concentrations tested and sampling times**	**Result**	
Mammalian erythrocyte micronucleus test OECD 474	Rat (SD) Male and female 5/sex/group	0, 150, 300, 600 mg/kg bw/d in males 0, 50, 100, 200 mg/kg bw/d in females Once by oral gavage 24 hours- all dose groups, vehicle and positive control. 48 hours- vehicle and highest dose for each sex. In a pre-test toxicity study, one male rat died at 600 mg/kg and 4/5 females died at 400mg/kg. For these reasons, the lower doses for female were used in the main study.	Clinical observations- male rats had tremors at 4 hours post dose at 300 and 600 mg/kg bw/d and soft stool at all three doses of S-1563. Tremor was also noted in female rats at 4 hours post treatment with 200 mg/kg bw/d. **24 h** (all groups) - neither sex showed any significant change in the percentage of PCEs (range 45.4- 62.2% in males and females across control and treated). PCEs with micronuclei did not alter significantly or dose dependently compared to controls in either sex (range 0.08-0.20 % across females and males). **48 h** (high dose and controls only) – males showed no change in PCEs or PCEs with micronuclei. In contrast, the percentage of PCEs in females did decline at 200 mg/kg bw/d (40.0 versus 54.4% in controls). This demonstrated that the test substance reaches the target tissue i.e. the bone marrow. The decline in PCEs was not accompanied by any changes in micronuclei. Positive control (cyclophosphamide) decreased the number of PCEs and induced micronuclei which satisfied the laboratory's criteria for validation.	Kitamoto (2010)
UDS assay OECD 486	Rat (SD) Male and female 3/sex/group	0, 100, 200 mg/kg bw/d in females 0, 300, 600 mg/kg bw/d in males All dose levels were investigated at 2 and 16 hours	**2 and 16 hours**- there were no significant differences in mean net nuclear grains or percentage of cells in repair at either exposure times or across sexes. Positive control at both time points showed enhanced net nuclear grains and cells in repair in both sexes of the rat. The results were in accordance with the	Tanaka (2010)

		post treatment. Once by oral gavage	laboratory's criteria.	

3.10.1 Non-human information

3.10.1.1 In vitro data

The bacterial reverse mutation assay and mammalian gene mutation assay with Chinese hamster V79 cells produced negative results in the presence and absence of S9. In contrast, a marginal positive result for structural aberrations was reported in the chromosome aberration assay in the presence of S9 at 100 µg/ml and above. This result indicates that 1R-trans-Z-momfluorothrin has genotoxic potential *in vitro*.

3.10.1.2 In vivo data

Two *in vivo* studies have been evaluated to determine the potential for 1R-trans-Z-momfluorothrin to induce cytogenetic damage and DNA repair in rats. No evidence of micronucleus formation was observed in either sex of the rat in the mammalian erythrocyte micronucleus test. To ensure this is a true negative result, it should be demonstrated that the test substance reaches the target tissue i.e. the bone marrow. The decline in PCEs in females at 48 hours supports this criterion and there is data from the toxicokinetic studies to suggest it is detected at low levels in the bone marrow following oral dosing (Mikata K, 2010).

In the UDS assay, there was also no evidence of enhanced DNA repair caused by 1R-trans-Z-momfluorothrin.

Overall, the results of these studies provide reassurance that 1R-trans-Z-momfluorothrin has no *in vivo* mutagenic potential on somatic cells.

No studies on germ cells have been submitted and this is justified based on the negative effects *in vitro* and *in vivo* with 1R-trans-Z-momfluorothrin.

3.10.2 Human information

No data available.

3.10.3 Other relevant information

None available.

3.10.4 Summary and discussion of mutagenicity

Mutagenicity of 1R-trans-Z-momfluorothrin has been investigated in 5 studies. Two *in vitro* studies were negative for mutagenicity (reverse mutation in bacteria and gene mutation in mammalian cells) but the chromosome aberration assay with metabolic activation did show a marginal increase in structurally aberrant chromosomes. *In vivo*, the assays showed that 1R-trans-Z-momfluorothrin

did not induce micronuclei or DNA damage. Based on the weight of evidence approach, 1R-trans-Z-momfluorothrin is not mutagenic in the test systems used.

3.10.5 Comparison with criteria

Under CLP, substances can be classified as a Cat 1A, 1B or 2 germ cell mutagen. For Category 1 A and B, the substance should be known to induce heritable changes or regarded as if they induce heritable changes in germ cells of humans or produce positive results *in vivo* somatic cell tests in combination with evidence that the substance has the potential to cause mutations in germ cells. There are no human data or positive results *in vivo* to suggest that 1R-trans-Z-momfluorothrin causes heritable mutations and therefore is not a Cat 1A or Cat 1B mutagen.

To attain category 2 under CLP, the substance needs to show positive results in mammals and /or in some cases *in vitro* experiments. In the case of 1R-trans-Z-momfluorothrin, the combined findings from all 5 studies do not support classification for mutagenicity in Category 2. Overall, it is concluded that 1R-trans-Z-momfluorothrin should not be classified for mutagenicity.

3.10.6 Conclusions on classification and labelling

Not classified – conclusive but not sufficient for classification

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter's proposal

Three GLP compliant *in vitro* and two *in vivo* genotoxicity studies, all conducted according to their respective OECD TG, were included in the CLH report to evaluate the potential of 1R-trans-Z-momfluorothrin to cause mutagenicity. The bacterial reverse mutation assay (Ames test, OECD TG 471) and the mammalian gene mutation assay with Chinese hamster V79 cells (OECD TG 476) produced negative results in the presence and absence of metabolic activation. The *in vitro* chromosome aberration assay in Chinese hamster lung cells (OECD TG 473) was marginally positive for structural aberrations in the presence of metabolic activation at and above 100 μg/mL.

In the *in vivo* mammalian erythrocyte micronucleus test (OECD TG 474) no evidence of micronucleus formation was observed in rats following oral gavage exposure to 1R-trans-Z-momfluorothrin. The decline in PCEs in females at 48 hours indicated that 1R-trans-Z-momfluorothrin had reached the bone marrow. Furthermore, data from the toxicokinetic studies suggested detection at low levels in the bone marrow following oral dosing.

In the UDS assay (OECD TG 486), there was also no evidence of enhanced DNA repair in rat liver following oral gavage exposure to 1R-trans-Z-momfluorothrin.

Overall, the DS concluded that 1R-trans-Z-momfluorothrin has no *in vivo* mutagenic potential on somatic cells, and proposed no classification for mutagenicity.

Comments received during public consultation

No specific comments were received during PC, but 2 MSCAs agreed in general terms on the proposed classification for human health hazards.

Assessment and comparison with the classification criteria

1R-trans-Z-momfluorothrin tested negative in two *in vitro* assays (a bacterial mutation assay, and a mammalian gene mutation assay). It was mildly positive in another *in vitro* assay (a chromosome aberration assay), yet it was negative in an *in vivo* micronucleus test. In addition, it

was also found negative in an *in vivo* UDS assay. RAC therefore supports the conclusion of the DS that **1R-trans-Z-momfluorothrin should not be classified for mutagenicity**.

3.11 Carcinogenicity

Table 19: Summary table of relevant carcinogenicity studies

Method	Dose levels	Observations and remarks (effects of major toxicological significance)	Reference
Oral- dietary intake Mouse, CD-1 Males and females 52/sex/group (main group) 12/sex/group (satellite group) OECD 451 Study compliant with GLP and OECD guidelines	0, 600, 2500, 5500 ppm for 52 weeks and 78 weeks (satellite and main groups respectively) Males - 0, 72, 308, 639mg/kg bw/d (main) 0, 74, 308, 672 mg/kg bw/d (satellite) Females - 0, 99, 427, 853 mg/kg bw/d (main) 0, 111, 433, 934 mg/kg bw/d (satellite) S-1563 Purity: 95.7%	There were no significant increases in mortality in any dose group. Nor were there any significant treatment related tumours in the mice in this study. All dose levels are above the guidance value for classification of non-neoplastic effects (Guidance value for classification of non-neoplastic effects: ≤25mg/kg bw/d). Treatment related effects include: **Satellite groups:** hepatocellular hypertrophy in females. **Main groups:** reduced body weight and body weight gain, decreased food consumption, increased liver weight accompanied by hypertrophy mainly centrilobular at minor severity, enhanced single cell necrosis in the liver and a hepatocellular brown pigment was recorded. NOAEL*: 600 ppm (72 mg/kg body weight/day in males and 99 mg/kg body weight/day in females).	Rached (2012a)
Oral- dietary intake Rat, Wistar (SPF)) Males and females 51/sex/ group OECD 451	0, 200, 500, 1500, 3000 ppm for 104 weeks Males- 0, 9.5, 23, 73, 154 mg/kg bw/d Females- 0, 11.1, 28, 88, 182 mg/kg bw/d S-1563 Purity: 95.7%	Males- Non-neoplastic findings **3000 ppm**- decreased mean body weight and body weight gain, increased relative liver weight (65.6%), liver cell hypertrophy, brown pigment, biliary cysts and cystic degeneration. Thyroid cell hypertrophy as a secondary effect to liver changes, brown pigment in the kidney and diffuse acinar hypertrophy in the mandibular glands were reported but not a concern. **1500 ppm**- decreased body weight and body weight gain, biliary cystic degeneration, brown pigment of the liver and kidneys, hepatocyte hypertrophy and diffuse acinar hypertrophy in the mandibular glands. The latter is considered nonadverse as it did not increase in severity from sub chronic and chronic studies). **500 ppm-** no adverse effects **200 ppm-** no adverse changes **Males-neoplastic findings** Prior to scheduled necropsy, 3 decedents in the top dose group had liver tumours which were treatment related. **3000 ppm**- liver nodules (13/51) and hepatic cysts (6/51) at necropsy. Increased incidence of eosinophilic cell foci (20/51), adenoma (8/51), carcinoma (9/51) and combined adenoma and	Rached (2012b)

carcinoma (17/51),

Incidence of neoplasm with ascending dose

Hepatocellular adenoma-2%, 0%, 4%, 8% and 16%

Hepatocellular carcinoma- 0%, 0%, 0%, 8% and 18%

There is a clear dose related trend and significance is attained at the top dose which is also above the historical control range provided.

Females- Non-neoplastic findings

3000 ppm- decrease in body weight and body weight gain and increased relative liver weight (46.1%), were significant. In addition, hepatic cell hypertrophy, brown pigment and biliary cysts were reported in the liver.

Brown pigment recorded in the kidney and diffuse acinar hypertrophy in the mandibular glands were observed but all considered non adverse as they have not progressed in severity from the chronic studies.

1500 ppm- decrease in body weight and body weight gain and increased salivary gland diffuse acinar hypertrophy (not adverse).

500 ppm- decrease in body weight and body weight gain.

200 ppm- no adverse changes.

Female- neoplastic findings

3000 ppm- hepatic cysts (13/51) at necropsy. Eosinophilic foci (9/51), adenoma (4/51), carcinoma (1/51) and combined adenoma and carcinoma (5/51).

Incidence of neoplasm with ascending dose

Hepatocellular adenoma- 0%, 0%, 2%, 2% and 8%

Hepatocellular carcinoma- 0%, 0%, 0%, 0% and 2%

Laboratory historical control data on lesions in Wistar rat recorded from 20, 104 week dietary studies conducted between 1981 and 2009

Organ/ findings	Males			Females		
	Mean (%)	Min (%)	Max (%)	Mean (%)	Min (%)	Max (%)
Liver						
Hepatocyte hypertrophy	2.57	0.00	20.00	2.93	0.00	27.00
Biliary cysts	1.22	0.00	8.00	3.45	0.00	14.00
Cystic degeneration	1.03	0.00	10.13	0.46	0.00	4.00
Eosinophilic cell foci	6.55	0.00	44.00	7.42	0.00	56.00
Hepatocellular adenoma	2.37	0.00	8.00	2.54	0.00	10.20
Hepatocellular carcinoma	0.26	0.00	2.80	0.26	0.00	2.00

		Thyroid glands						
		Follicular cell hypertrophy	5.60	0.00	36.00	3.57	0.00	24.74

* As cited in the CAR

3.11.1 Non-human information

3.11.1.1 Carcinogenicity: oral

Two GLP compliant studies have been submitted and are in accordance with OECD test guideline 451.

Mouse

The dietary study in mice over 78 weeks did not produce a carcinogenic response. Liver abnormalities in both males and females at 2500 and 5500 ppm (308/427 (male/female) and 639/853 (male/female) mg/kg bw/d respectively), decreased body weight gain in males at 2500 and 5500 ppm and decreased food consumption at 5500 ppm are considered to be toxicologically significant.

Rat

In the rat study, mean body weight was significantly decreased in males treated with 1500 (11%) and 3000 ppm (21%) and female rats treated with 500 (5%), 1500 (13%) and 3000 ppm (19%). This was accompanied by a decrease in mean body weight gain in both sexes at these doses. The decreases in body weight and body weight gain are concluded to be treatment related. Mortality was monitored daily during the study and the rates of mortality were similar across controls and treated groups. Three males treated with 3000 ppm S-1563 that died prematurely did have liver tumours, however, that were interpreted to be treatment related. Other findings included increased relative liver weight in both sexes at 3000 ppm (65.6% in males and 46.1% in females), liver nodules in 13/51 male rats at 3000 ppm and hepatic cysts in both sexes at 3000 ppm (6/51 males and 13/51 females) at necropsy.

Non-neoplastic hepatic lesions included hypertrophy, brownish pigment and biliary cysts; these all occurred in both sexes at 3000 ppm and were statistically significant. The incidence of hypertrophy was 14/51 and 10/51 in males and females respectively, the brownish pigment was present in the liver of 36/51 males and 18/51 females whilst the incidence of biliary cysts was 8/51 and 17/51 for males and females respectively. Male rats were also reported to have a significant increase in cystic degeneration in the liver at 1500 (7/51) and 3000 ppm (7/51).

Hyperplastic and neoplastic findings were reported at the highest dose in the liver and included an increased incidence of eosinophilic cell foci (20/51), adenoma (8/51), carcinoma (9/51) and combined incidence of adenoma and carcinoma (17/51) in male rats and eosinophilic foci (9/51), adenoma (4/51), carcinoma (1/51) and combined incidence of adenoma and carcinoma (5/51) in females.

All the non-neo- and neoplastic liver effects reported in male and female rats were in excess of the mean value from historical control data but did not always exceed the maximum value quoted. In male rats, the incidences of adenoma, carcinoma, cystic degeneration, biliary cysts and hypertrophy, but not eosinophilic foci, were outside the ranges reported for historical controls. In female rats, only the incidence of biliary cysts was outside the historical range values stated.

Other effects that were recorded in the rat include diffuse follicular cell hypertrophy in the thyroid gland in males at 3000 ppm, brownish pigment in the kidney in both sexes at 3000 ppm, diffuse acinar hypertrophy in the mandibular glands in both sexes at 1500 ppm and 3000 ppm. None of these findings are significant as they have not increased in severity from the chronic studies, are considered secondary to the liver effects or are not associated with other indicators of injury.

Overall, the incidence of adenoma/carcinoma in the rats is a concern but available evidence suggests that the tumour formation is not a result of genotoxic activity (see section 4.9). To address the potential non-genotoxic mechanism and its relevance to humans, a series of additional mode of action studies were undertaken (see section 4.10.3).

3.11.1.2 Carcinogenicity: inhalation

No information available.

3.11.1.3 Carcinogenicity: dermal

No information available.

3.11.2 Human information

No information available.

3.11.3 Other relevant information

The UK CA has had extensive discussions with the applicant regarding the mode of action for the liver tumours seen in rats treated with 1R-trans-Z-momfluorothrin. The applicant provided a document (See Annex 1) outlining their position and the mechanistic data available to justify the proposed mode of action: i.e. that the increased liver tumours seen in rats treated with 1R-trans-Z-momfluorothrin were linked specifically to a mechanism involving activation of the constitutive androstane receptor (CAR) that was not considered relevant to humans (see also the review by Elcombe *et al*, 2014). The mechanistic studies underpinning this position are summarised briefly below.

A number of *in vivo* and *in vitro* studies were submitted to address the mode of action responsible for the occurrence of liver tumours. These studies are not claiming GLP compliance but abided by GLP principles for these non-guideline tests.

In vitro studies with rat hepatocytes

Table 20: In vitro studies with rat hepatocytes

Test system	Conditions	Remarks	Result and conclusion	Reference
Study to investigate gene expression specific to CAR activation Primary cultured hepatocytes sourced from 1 male Wistar rat and transfected with	Cultured hepatocytes transfected with siRNA (CAR) or siRNA (control) were exposed for days to:	The RNA interference technique was employed to reduce CAR mRNA levels in rat hepatocytes and thus examine the importance of CAR	In cells treated with CAR-siRNA, both exposure to 1R-trans-Z-momfluorothrin and phenobarbital reduced CAR mRNA levels, resulting in a significant reduction in	Okuda, 2012a

either: 1) siRNA for CAR (1 µg) + MA Tra-si reagent (1 µL) + serum/antibiotic free medium (200 µM) 2) "negative" control siRNA (1 µg) + MA Tra-si reagent (1 µL) +serum/antibiotic free medium (200 µM) 3) Untreated group – not exposed to transfection mixture Analysis and quantification of isolated mRNA (CAR, CYP 2B1/2 and GAPDH (used to normalize levels of CAR and CYP 2B1/2)) was conducted using quantitative real time PCR.	1R-trans-Z-momfluorothrin (S-1563): 100 µM Phenobarbital: 50 µM (positive control) Untreated hepatocytes were not exposed to either 1R-trans-Z-momfluorothrin or phenobarbital.	in the CYP2B induction stimulated by 1R-trans-Z-momfluorothrin and phenobarbital.	the magnitude of induction of CYP2B1/2 mRNA levels. 1R-trans-Z-momfluorothrin, like phenobarbital, induced CYP2B1/2 mRNA in rat hepatocytes and this is mediated via activation of CAR (which is inhibited by treatment with siRNA).	
Study to investigate induction of mRNA coding for cytochrome P450 Primary cultured hepatocytes sourced from male Wistar rats Analyses of isolated mRNA samples were made by quantitative real-time PCR (one sample per animal per treatment). Average taken across the results of all animals at each concentration.	Hepatocytes were exposed for 72 hours to either: 1R-trans-Z-momfluorothrin (S-1563): 0, 1,5, 10, 50, 100, 500 and 1000 µM 0 and 5-500 µM: 4 animals 1 and 1000 µM: 3 animals Or Phenobarbital: 0, 500 or 1000 µM (5, 2 or 3 animals, respectively)	cDNA was prepared from total RNA by reverse transcription polymerase chain reaction (RT-PCR). Analysis and quantification of isolated mRNA (CYP2B1/2, CAR and GAPDH (used to normalize levels of CAR and CYP 2B1/2)) was conducted using quantitative real time PCR	*1R-trans-Z-momfluorothrin:* 3-fold increase in CYP2B1/2 levels at 50 µM, moderate but insignificant changes at 100 and 500 µM compared with control *Phenobarbital:* 45-fold increase in CYP2B1/2 levels compared with control 1R-trans-Z-momfluorothrin induced CYP2B1/2 in cultured rat hepatocytes.	Okuda, 2013
Induction of replicative DNA synthesis in rat hepatocytes- Primary cultured	1R-trans-Z-momfluorothrin (S-1563): 0, 1, 5, 10, 50, 100, 500 and 1000 µM	*Cell proliferation (measured as replicative DNA synthesis)*	Phenobarbital was used for comparison purposes as a CAR activator. HGF was used successfully to validate the experiment	Okuda 2013

hepatocytes sourced from male Wistar rats. To measure DNA replication, 5-bromo-2'-deoxyuridine (BrdU) was added to the media of cells of all treatment groups during the last 24 hrs of the 48 hr treatment period. One sample per animal per treatment was reported and an average taken across the results of all animals at each concentration.	0-50 µM: 6 animals 100-1000 µM: 3 animals Phenobarbital: 0, 500 or 1000 µM (8, 4 or 4 animals respectively) HGF: 0, 10 and 100 ng/ml (4 animals at all doses) Cells were exposed for 48 hours before harvesting for analysis.	There was increased replicative DNA synthesis in cultures treated with 1R-trans-Z-momfluorothrin and phenobarbital. With HGF (Hepatocyte growth factor), there was a significant concentration dependent increase in replicative DNA synthesis (max 4-fold)	as human hepatocytes cells were responsive to this mitogenic factor. No explanation was provided for the decrease in replicative DNA synthesis seen at the higher concentrations of 1R-trans-Z-momfluorothrin.	

In the first *in vitro* study, fresh hepatocytes were isolated from untreated male Wistar rats and incubated in serum-free conditions with CAR siRNA (CAR short interfering RNA 100 nM) for 4-hours (Okuda, 2012a). The medium was then changed to a standard hepatocyte serum-based medium supplemented with 1R-trans-Z-momfluorothrin or phenobarbital. Two controls were established, completely untreated hepatocytes and hepatocytes treated with the siRNA (negative control). After a further 2-day incubation period, total RNA was isolated, reverse transcribed to cDNA and assayed for CAR and CYP2B1/2 using real-time quantitative PCR (Polymerase Chain Reaction). The intended effect of the CAR siRNA is to block transcription of CAR mRNA and decrease the amount of functional CAR.

A statistically significant decrease in CAR mRNA (19% of negative controls) was observed in hepatocytes treated with CAR siRNA and Momflurorothrin at 100µM. When hepatocytes pre-treated with CAR siRNA were induced with 1R-trans-Z-momfluorothrin or phenobarbital, statistically significant decreases in CYP2B1/2 mRNA were observed (32% and 33% of negative control, with 1R-trans-Z-momfluorothrin and phenobarbital respectively). Overall, this study indicates that the CAR is involved in the induction of CYP2B1/2 mRNA.

In a second *in vitro* study, the effects of 1R-trans-Z-momfluorothrin and phenobarbital on CYP 2B1/2 mRNA expression and DNA replication, in rat hepatocytes were investigated (Okuda, 2013).

For CYP2B1/2 mRNA, primary cultures of rat hepatocytes were incubated for 48 hours with 1R-trans-Z-momfluorothrin or phenobarbital. At the end of this period, the cells were harvested for mRNA, which was then reverse transcribed and subject to real time quantitative PCR. To determine replicative DNA synthesis, the hepatocytes were incubated for 48-hours in 5% foetal bovine serum (FBS) medium containing 0.5 ng/ml epidermal growth factor (EGF) and either phenobarbital, 1R-trans-Z-momfluorothrin or hepatocyte growth factor (HGF) for 48 hours. BrdU was added 24 hours before assay termination and proliferation rate calculated compared to untreated controls.

Compared to controls, CYP2B1/2 mRNA was increased to a maximum of 3-fold in rat hepatocytes treated with 50 µM and decreased at higher doses. Treatment with phenobarbital increased CYP2B1/2 mRNA levels to 45-fold of controls. Replicative DNA synthesis was statistically significantly increased in rat hepatocytes treated with 1R-trans-Z-momfluorothrin at concentrations

of 5 and 10 µM (1.6 and 1.8-fold) and in phenobarbital treated rat hepatocytes at 500 µM and above (max 1.4-fold).

In vivo mechanistic studies in rats

Males

In the cancer study, dietary exposure of male rats with approx. 75 and 150 mg/kg/day 1R-trans-Z-momfluorothrin resulted in increased incidences of liver adenoma and carcinoma. The following data from short term studies provide a mechanistic basis for these findings.

Table 21: In vivo mechanistic studies in male rats

Method	**1R-trans-Z-momfluorothrin (S-1563)**	**Endpoint**	**Reference**
Male rat, Wistar 10/group Oral, dietary CYP mRNA levels: Total RNA was extracted from a liver fraction and reverse transcribed to generate cDNA. Using primers specific to CYP2B1/2, the cDNA for the enzyme was quantified by real-time PCR. CYP activity: S9 fractions were harvested from the rats. CYP2B activity was measured as pentoxyresorufin O-depentylase (Deguchi *et al*, 2009). Hypertrophy: One section of the left lateral lobe and right medial lobe were fixed, stained and examined by light microscopy. Cell proliferation: Mini-pumps were inserted into the subcutaneous back region of the rats and BrdU released at a rate of 10µl/hour for 4 or 7 days preceding euthanasia	0- 3000ppm 0-137 mg/kg bw/d 7 days	**CYP2B activity**: maximum fold change was 2.8 compared to controls **Hypertrophy:** 2/10 animals with hypertrophy at highest dose **Cell proliferation**: maximum fold change was 2.5 compared to controls	Okuda, 2012b
	0 and 3000 ppm 0 and 147 mg/kg bw/d 7 days	**CYP mRNA levels**: maximum fold change was 17.8 compared to controls **Hypertrophy:** 3/10 animals with hypertrophy at highest dose **Cell proliferation:** maximum fold change was 1.4 compared to controls	Okuda, 2012c
	0- 6000 ppm 0- 258 mg/kg bw/d 7 days	**CYP2B activity:** maximum fold change was 10.8 compared to controls **Hypertrophy:** 10/10 animals with hypertrophy at highest dose	Okuda, 2012d
	0 and 3000 ppm 0 and 175 mg/kg bw/d 14 days	**CYP mRNA levels:** increased 16.4-fold compared to controls **Hypertrophy:** 4/10 animals with hypertrophy at highest dose but high incidence in control group (3/10). **Cell proliferation:** increased 1.8-fold compared to controls	Okuda, 2012c
	0, 3000 and 6000 ppm 0, 166 and 340 mg/kg bw/d 14 days	**Hypertrophy:** 10/10 animals with hypertrophy at highest dose	Okuda 2012d

Females

In the cancer study, dietary exposure of female rats with approx. 180 mg/kg/day S-1563 resulted in a small increased frequency in the incidence of hepatocellular adenoma. The following data from short term studies provide a mechanistic basis for this finding.

Table 22: In vivo mechanistic studies in female rats

Method	**1R-trans-Z-momfluorothrin (S-1563)**	**Endpoint**	**Reference**
Female rat, Wistar10/group Oral, dietary CYP activity: S9 fractions were harvested from the rats. CYP2B activity was measured as pentoxyresorufin O-dependentylase (Deguchi *et al*, 2009). Hypertrophy: One section of the left lateral lobe and right medial lobe were fixed, stained and examined by light microscopy. Cell proliferation: Mini-pumps were inserted into the subcutaneous back region of the rats and BrdU released at a rate of 10µl/hour for the 4 days preceding euthanasia	0- 3000ppm 0- 150.9 mg/kg bw/d 7 days	**CYP2B activity:** maximum fold change was 6.2 compared to controls **Hypertrophy:** 3/10 animals with hypertrophy at highest dose **Cell proliferation**: increased 2.8-fold compared to controls	Okuda, 2012e
	0 and 3000ppm 0- 163.8 mg/kg bw/d 14 days	**CYP2B activity:** an increase of 6.9-fold compared to controls **Hypertrophy:** 0/10 animals with hypertrophy at highest dose **Cell proliferation:** 1.7-fold increase compared to controls	Okuda, 2012e

In vivo effects- mouse

A range of comparable studies were reported in the mouse. They showed CYP 2B induction, increased hepatocellular hypertrophy and proliferation over 7 and 14 days at 5500 ppm S-1563 in the diet (equivalent to the highest dose in the carcinogenicity study) (Yamada, 2012a, b and c). There was also a slight increase in CYP4 induction, but electron microscopy did not show an accompanying increase in peroxisome proliferation. Although these results appear to indicate that 1R-trans-Z-momfluorothrin can produce CAR activation in the mouse, the exposure conditions employed in the mouse carcinogenicity study did not produce an increased frequency of liver tumours in this species. A full summary of these data are provided in Annexes IV and V of Annex I to the report.

In vitro studies with human hepatocytes

Cultures of human hepatocytes from up to a total of 10 donors were employed to investigate the relevance of the proposed mode of action to humans. These non-standard experiments indicated that human hepatocytes respond to 1R-trans-Z-momfluorothrin treatment with an increase in CAR activation but not increased cell proliferation. Similar findings were seen with phenobarbital.

However, the hepatocytes did show a proliferative response to HGF, showing that they did have the potential to produce such a response.

These *in vitro* studies are of critical importance, as they provide the only direct evidence supporting the view that 1R-trans-Z-momfluorothrin does not present a hepatocarcinogenic hazard to humans.

Table 23: In vitro studies with human hepatocytes

Test system	**Treatment conditions (no. of donors)**	**Results**	**Remarks**	**Reference**
Expression of CYP2B6 mRNA in human hepatocytes Primary cultures of human hepatocytes derived from 10 donors (5 males and 5 females). Human donors ranged from 10 months to 80 years old and were Caucasian (9/10) and Hispanic (1/10). Analyses of isolated mRNA samples were made by quantitative real-time PCR (one sample per donor per treatment). Average taken across the results of all donors at each concentration.	1R-trans-Z-momfluorothrin (as S-1563) :0 (5), 1 (3), 5 (5), 10 (4), 50 (5), 100 (5), 500 (5) and 1000 (1) µM Phenobarbital: 0 (7) and 1000 µM (7) Cells were exposed for 48 hours before harvesting for analysis.	*CYP2B6 mRNA induction* 1R-trans-Z-momfluorothrin S-1563: significant increases were seen at 100 and 500 µM (1.7 and 1.8-fold, respectively) compared with control. Smaller increases were seen at 50 and 1000 µM. Phenobarbital: significant 4.8 fold increase in CYP 2B6 mRNA compared with control	The lowest number of donors were used at the extremes of low and high doses and do not detract from the outcome of the study. A trend towards increased CYP2B6 was observed from 0 to 500µM. The increases seen with 1R-trans-Z-momfluorothrin were of a comparable magnitude to those seen in studies with rat hepatocytes. However, the responses to phenobarbital were much greater in rat hepatocytes.	Okuda, 2013
Induction of replicative DNA synthesis Primary cultures of human hepatocytes derived from 10 donors (5 males and 5 females). Human donors ranged from 10 months to 80 years old and were Caucasian (9/10) or Hispanic (1/10). To measure DNA replication, 5-bromo-2'-deoxyuridine (BrdU) was added to the media of cells of all treatment groups	1R-trans-Z-momfluorothrin (as S-1563): 0 (8), 1 (8), 5 (8), 10 (8), 50 (8), 100 (5), 500 (5) and 1000 (5) µM Phenobarbital: 0 (10), 500 (8) and 1000 (6) µM HGF: 0 (10), 10 (5) and 100 (10) ng/ml Cells were exposed for 48 hours before	*Cell proliferation (measured as replicative DNA synthesis)* There was no increased replicative DNA synthesis in cultures treated with 1R-trans-Z-momfluorothrin or phenobarbital. On the other hand, decreased replicative DNA synthesis was observed at 100-1000 µM 1R-trans-Z-momfluorothrin.	Phenobarbital was used for comparison purposes as a CAR activator. HGF was used successfully to validate the experiment as human hepatocytes cells were responsive to this mitogenic factor. No explanation was provided for the decrease in replicative DNA synthesis seen at the higher concentrations of 1R-trans-Z-momfluorothrin.	Okuda, 2013

during the last 24 hrs of the 48 hr treatment period. One sample per donor per treatment was reported and an average taken across the results of all donors at each concentration.	harvesting for analysis.	With HGF, there was a significant concentration dependent increase in replicative DNA synthesis.		

In an *in vitro* study, the effects of 1R-trans-Z-momfluorothrin and phenobarbital were investigated on CYP 2B6 mRNA expression and DNA replication in human hepatocytes (Okuda, 2013).

For CYP2B6 mRNA, cultures of human hepatocytes were incubated for 48 hours with S-1563 or phenobarbital. At the end of this period, the cells were harvested for mRNA, which was then reverse transcribed and subject to real time quantitative PCR. To determine replicative DNA synthesis, the hepatocytes were incubated for 48-hours in medium containing 0.5 ng/ml EGF and either phenobarbital, S-1563 or HGF for 48 hours. BrdU was added 24 hours before assay termination and proliferation rate calculated compared to untreated controls.

Compared to controls, CYP 2B6 mRNA was increased in a concentration dependent manner with significant increases at 100 and 500 µM S-1563, 1.7 and 1.8-fold respectively. Treatment with phenobarbital increased CYP2B6 mRNA levels to 4.8-fold of controls. Replicative DNA synthesis was significantly increased in human hepatocytes by 1.6- and 3.7-fold when treated with HGF (10 and 100 ng/ml). In contrast, S-1563 and phenobarbital did not increase DNA synthesis compared to controls.

Consideration of other potential mechanisms

In determining a mode of action for a substance, other potential mechanisms should be discounted. Based on the results from the mutagenicity assays, 1R-trans-Z-momfluorothrin was not genotoxic and therefore a non-genotoxic mode of action is plausible. These include cytotoxicity, activation of CAR or PPARα, porphyria and hormonal perturbation. Throughout the dossier, there has been no suggestion of hormonal perturbation, porphyria or increased iron deposition. Hepatocellular toxicity (e.g. fatty liver and necrosis) has not been reported in any of the short term or long term studies with 1R-trans-Z-momfluorothrin so cytotoxicity is not a contributing factor. Electron microscopy in rodents at 7, 14 and 90 days has shown that cellular ultrastructure changes are confined to augmentation of the smooth endoplasmic reticulum and not peroxisome proliferation as would occur for PPARα stimulation.

To discount activation of other receptors, a study by Lake in 2009 (full study not available, see Annex) showed that only CYP2B1/2 was induced, consistent with CAR activation, but there was no induction of CYP1A2, 3A1, 3A2 or 4A1 which are more specific to the activation of other receptors: aryl hydrocarbon receptor (AhR), pregnane X receptor (PXR) and PPARα, respectively.

Taken together, these results suggest that 1R-trans-Z-momfluorothrin does not act via activation of PPARα, AhR or PXR to provoke a hepatocarcinogenic response in rats, nor does it produce a consistent cytotoxic response, hormonal perturbations or porphyria that could also explain its carcinogenicity in this species. The most plausible explanation is that 1R-trans-Z-momfluorothrin acts in the rat by activation of CAR, which results in altered gene expression specific to CAR activation and subsequently increased cell proliferation and formation of altered hepatic foci.

Data gaps, uncertainties and inconsistencies

There is no experimental evidence available for several events that may be associated with the CAR-mediated mechanism of liver cancer induction in rats. No evidence has been seen of decreased apoptosis, inhibition of gap junction intercellular communication or altered epigenetic changes. However, as these are not regarded as key events, they are not critical data gaps.

It is unclear why no proliferative response to 1R-trans-Z-momfluorothrin was seen in cultured human hepatocytes. With no explanation, there remains a possibility that the findings *in vitro* might not be relevant *in vivo*. However, some degree of reassurance is provided by the observation that a proliferative response was induced in human hepatocyte cultures by HGF. Thus, it was not that the cells were incapable of providing such a response *in vitro*.

The findings in mice are somewhat inconsistent. Although short term studies show a potential of 1R-trans-Z-momfluorothrin to activate CAR in this species, it did not induce a hepatocarcinogenic response. It is possible that the conditions employed in the mouse study were not optimised for a hepatocarcinogenic response and a different dosing schedule may have produced a positive result. However, any such finding would still have been considered of limited or no relevance to humans.

The applicant has proposed that the species difference is consistent with other pyrethroids, particularly a structural analogue, metofluthrin. Whilst the species differences have not been fully addressed, CAR activation remains a plausible mechanism based on all the available data in the rat.

Relevance to humans

As shown in table 24, there are only certain elements of the proposed mode of action for 1R-trans-Z-momfluorothrin-induced carcinogenicity in rats that are predicted to occur in humans.

Table 24: Relevance to humans

Key and associative events	**Evidence in rats**	**Evidence in humans**
Activation of CAR	Suggested through the *in vitro* study with RNAi for CAR and induction of CYP2B enzymes	Probable at high doses based on the induction of CYP2B *in vitro*
Altered gene expression	Only changes in CYP2B reported in this submission	None in this submission but does occur in other published studies
Induction if CYP2B as a marker for CAR activation	Experimental evidence *in vivo* and *in vitro* with cultured rat hepatocytes	Experimental evidence based on the induction of CYP2B *in vitro*
Hypertrophy	Experimental evidence *in vivo*	Possible based on published evidence in humans treated with anticonvulsant drugs
Increased hepatocellular proliferation	Experimental evidence *in vivo* and *in vitro* with cultured rat hepatocytes	Not predicted as not reported in cultured hepatocytes
Altered hepatic foci	Experimental evidence *in vivo*	Not predicted
Liver tumours	Yes	Not predicted
Inhibition of gap junctional intercellular communication	None in this submission	None in this submission

Altered DNA methylation/ epigenetic changes	None in this submission	None in this submission
Decreased apoptosis	None in this submission	None in this submission

Overall, the differences between rat and human data with 1R-trans-Z-momfluorothrin were consistent with other compounds with a CAR mediated mode of action (see Elcombe *et al*, 2014 for a detailed review).

Statement of confidence

Taking all the available data into consideration, there is clear evidence to support CAR activation in male and female rats following 1R-trans-Z-momfluorothrin treatment and this being a plausible mode of action for the formation of hepatic tumours in this species. It has been demonstrated that 1R-trans-Z-momfluorothrin causes CYP2B induction, hepatocyte hypertrophy and cell replication *in vivo* and *in vitro* for rats by CAR activation. This is consistent with the potential to cause cell foci and tumours in long term studies. In contrast, available data suggest that 1R-trans-Z-momfluorothrin is capable of inducing CYP2B6 and hypertrophy but then does not increase cell replication in human hepatocytes, which is a prerequisite for tumour formation. Overall, the CAR mechanism in rats is of limited relevance to humans.

3.11.4 Summary and discussion of carcinogenicity

The carcinogenicity study in the mouse was negative for all tumour types but in the rat, particularly males, an increase in the incidence of adenoma and carcinoma of the liver was seen at the top dose of 1R-trans-Z-momfluorothrin (3000 ppm S-1563 in the diet; equivalent to approx. 150 mg/kg S-1563 in males and 180 mg/kg S-1563 in females). In males, there was also a slight increase in the frequency of liver adenoma and carcinoma at 1500 ppm (75 mg/kg).

The findings in an extensive set of short term assays have indicated that 1R-trans-Z-momfluorothrin is unlikely to have acted as a genotoxic carcinogen. In contrast, there are mechanistic data available that point towards a non-genotoxic mechanism of carcinogenesis, driven initially by activation of the nuclear constitutive androstane receptor (CAR) and later by a proliferative response in the liver.

Specifically, the postulated mode of action involves the following sequence of key events:

1. Nuclear membrane receptor activation (CAR)
2. Altered gene expression specific to CAR activation (e.g. CYP2B)
3. Liver hypertrophy
4. Cell proliferation (which can be studied in vitro and in vivo)
5. Clonal expansion to generate altered liver cell foci
6. Increased liver adenoma/carcinoma

With 1R-trans-Z-momfluorothrin, activation of CAR was shown *in vitro* by the increase in CYP2B transcription which was attenuated in the presence of CAR specific siRNA. Three short-term *in vivo* studies in the rat further demonstrated that 1R-trans-Z-momfluorothrin had a dose-dependent potential to increase hepatic CYP2B levels. Cultured rat hepatocytes also showed increased CYP2B mRNA levels in response to 1R-trans-Z-momfluorothrin.

Hepatocellular hypertrophy was observed throughout the liver lobule in the original carcinogenicity study and at a comparable dose level (3000 ppm) in the mode of action studies. In addition, examination by electron microscopy of liver sections from male and/or female rats given 3000 ppm S-1563 for 1 or 2 weeks and 6000 ppm S-1563 for 3 months in the repeat dose study (Sommer, 2011b) revealed increased smooth endoplasmic reticulum which is a characteristic of enzyme inducers.

Data from an *in vitro* study showed 1R-trans-Z-momfluorothrin can induce hepatocellular replication: DNA synthesis being increased in cultured rat hepatocytes at 5 -10 μM. A proliferative response was also seen in short-term *in vivo* studies.

The carcinogenicity study reported eosinophilic foci in the livers of rats at 3000 ppm 1R-trans-Z-momfluorothrin with an incidence of 20/51 in males and 9/51 in females. This can be considered indicative of preneoplastic lesions. Finally, the incidence of adenoma and/or carcinoma was enhanced in both sexes of the rat but more markedly in males (17/51 versus 5/51 in females).

Studies with human hepatocytes from a variety of male and female donors have shown that 1R-trans-Z-momfluorothrin can similarly induce activation of human hepatic CAR, but that DNA replication (leading to hepatocellular proliferation) does not then follow. The findings with 1R-trans-Z-momfluorothrin are therefore consistent with the conclusion expressed in the recent detailed review of Elcombe *et al* (2014) that this mode of action exemplified by phenobarbital is considered to be qualitatively not plausible for humans.

Taking into consideration all available evidence, it is concluded that although carcinogenic in the rat, 1R-trans-Z-momfluorothrin does not present a significant carcinogenic hazard to humans. The carcinogenicity in rats appears to proceed via CAR activation which is a mechanism with very limited or no relevance to humans.

3.11.5 Comparison with criteria

In accordance with the criteria under CLP, classification in category 1A for carcinogenicity is not justified as there is no evidence of 1R-trans-Z-momfluorothrin having caused cancer in humans. It is therefore necessary to decide whether 1R-trans-Z-momfluorothrin fulfils the criteria for category 1B, category 2 or does not require classification.

To attain category 1B there should be sufficient evidence for carcinogenicity in experimental animals. In the case of 1R-trans-Z-momfluorothrin, the animal data are only limited rather than sufficient due to tumour incidence being restricted to one species, in a single tissue, with no evidence of a genotoxic mode of action. A carcinogenic response was seen in rats, but not mice.

Ordinarily, this level of evidence in experimental animals would be sufficient for 1R-trans-Z-momfluorothrin to be classified in Category 2 for carcinogenicity, but taking into account the supporting *in vivo* and *in vitro* mechanistic studies, it can be argued that 1R-trans-Z-momfluorothrin causes tumours in rats by a mechanism that is not relevant to humans. In this instance, therefore, no classification for carcinogenicity seems most appropriate.

3.11.6 Conclusions on classification and labelling

Not classified – conclusive but not sufficient for classification.

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

No classification for carcinogenicity was proposed by the DS. Two GLP compliant carcinogenicity studies (OECD TG 451) were summarised in the CLH report: a 104-week dietary study in rats and a 78-week dietary study in mice. In addition, several *in vitro* and *in vivo* studies investigating the mechanism of tumour formation in the rat were summarised (all non-GLP, non-guideline) and a Human Relevance Framework (HRF) analysis was performed, in order to establish the human relevance of the tumours induced by 1R-trans-Z-momfluorothrin (see also Annex I to CLH report).

1R-trans-Z-momfluorothrin at dose levels of 600, 2500 or 5500 ppm in the diet did not produce a carcinogenic response in the 78-week mouse study (which included a 52-week exposure satellite group). Treatment-related adverse effects were seen from 2500 ppm and included decreases in body weight and body weight gain, decreased food consumption, increased liver weight accompanied by hepatocellular hypertrophy (mainly centrilobular), and enhanced single cell necrosis and brown pigmentation in the liver (see Table below).

Table Main findings in 78-week mouse study

	Males				**Females**			
Nominal dietary concentration (ppm)	**0**	**600**	**2500**	**5500**	**0**	**600**	**2500**	**5500**
Mortality (Main groups)	6/52	3/52	4/52	6/52	12/52	11/52	5/52	8/52
(Satellite groups)	1/12	1/12	0/12	1/12	0/12	2/12	1/12	1/12
Body weight, Week 78 (g)	51.64	50.94	45.54**	41.89**	34.52	34.03	32.64	33.03
Food consumption, weeks 1-78 (g/mouse/day; mean of means)	5.24	5.03	5.01	4.45	5.07	4.88	4.99	4.53
Organ weights								
Satellite groups (52-week Interim sacrifice)								
TERMINAL BODYWEIGHT (G)	49.5	53.0	44.4	41.8*	33.5	33.3	31.7	31.8
LIVER WT (G)	2.25	2.67	2.48	2.75*	1.54	1.67	1.85**	2.24**
(% BODYWT)	4.54	5.02	5.59**	6.57**	4.61	5.06	5.83**	7.03**
Main groups (78-week terminal sacrifice)								
TERMINAL BODYWEIGHT (G)	50.3	49.2	44.2**	41.0**	34.8	33.4	32.4*	32.7
LIVER WT (G)	2.33	2.61*	2.58*	2.97**	1.65	1.64	1.91**	2.35**
(% BODYWT)	4.65	5.37**	5.85**	7.22**	4.77	4.90	5.89**	7.15**
Histopathological Findings Incidence								
Satellite groups (52-week Interim sacrifice)								
LIVER (NO. OF ANIMALS EXAMINED)	(12)	(12)	(12)	(12)	(12)	(12)	(12)	(12)
HEPATOCELLULAR HYPERTROPHY	3	0	1	2	0	1	5*	9**
Main groups (78-week terminal sacrifice)								
LIVER (NO. OF EXAMINED ANIMALS)	(52)	(52)	(52)	(52)	(52)	(50)	(52)	(52)
HEPATOCELLULAR HYPERTROPHY	12	12	35**	32**	4	2	37**	47**
INCREASED SINGLE CELL NECROSIS	0	0	0	4	0	0	1	2

BROWNISH PIGMENT	0	0	0	13**	0	0	0	21**
ADENOMA#	7	7	1	3	0	0	0	1
CARCINOMA#	3	4	0	1	0	0	0	0

Values significantly different from control are : *p<0.05 and **p<0.01.
As reported in Annex I to CLH-report

In the rat carcinogenicity study, 1R-trans-Z-momfluorothrin was administered at dietary doses of 0, 200, 500, 1500 or 3000 ppm for 104 weeks. Treatment-related effects included decreased body weights (11% and 21% for males at 1500 and 3000 ppm, respectively, and 5%, 13% and 19% for females at 500, 1500 and 3000 ppm, respectively) and a corresponding decline in body weight gains, and increased relative liver weights (65.6% for males and 46.1% for females at 3000 ppm). Macroscopically, an increased incidence of liver nodules was seen in males at 3000 ppm (13/51) and of hepatic cysts in males (6/51) and females (13/51) at that dose level. Histopathologically, several non-neoplastic and neoplastic findings were reported for the liver, mainly at 3000 ppm in both sexes (see Table below).

The non-neoplastic liver findings included statistically significantly increased incidences of hypertrophy, brownish pigment, biliary cysts in both males and females and, in males only, cystic degeneration. The (pre-)neoplastic liver findings concerned increased incidences of eosinophylic foci, adenomas, carcinomas and combined adenomas and carcinomas in males and females at 3000 ppm. All increased incidences were statistically significant, with the exception of carcinomas in females. The incidences of tumours were also increased in males at 1500 ppm, but they were not statistically significant. Three high dose male rats died prematurely due to the presence of liver tumours. All non-neoplastic and (pre-)neoplastic liver effects reported in male and female rats were in excess of the mean value from historical control data but did not always exceed the maximum value reported (see Table below).

Other non-neoplastic effects considered to be of no concern included thyroid cell hypertrophy (considered secondary to the liver effects), brownish pigments in the kidney and diffuse acinar hypertrophy in the mandibular glands.

Table Non-neoplastic and neoplastic liver histopathological findings in the 104-week rat study (treatment-related findings are highlighted)

	Males						**Females**					
Nominal dietary conc. (ppm)	**0**	**200**	**500**	**1500**	**3000**	**HC#**	**0**	**200**	**500**	**1500**	**3000**	**HC#**
LIVER (NO. EXAMINED)	(51)	(51)	(51)	(51)	(51)	Mean (range)	(51)	(51)	(51)	(51)	(51)	Mean (range)
HEPATOCELLULAR HYPERTROPHY	1 *2%*	1 *2%*	0	5 *9.8%*	14** *27.5%*	*2.57% (0-20)*	0	0	0	3 *5.9%*	10** *19.6%*	*2.93% (0-27)*
BROWNISH PIGMENT					36 *70.6%*						18 *35.3%*	
BILIARY CYSTS					8 *15.7%*	*1.22% (0-8)*					17 *33.3%*	*3.45% (0-14)*
CYSTIC DEGENERATION				7 *13.7%*	7 *13.7%*	*1.03% (0-10.1)*						
EOSINOPHYLIC FOCI	0	2 *3.9%*	3 *5.9%*	3 *5.9%*	20** *39.2%*	*6.55% (0-44)*	2 *3.9%*	0	2 *3.9%*	5 *9.8%*	9* *17.6%*	*7.42% (0-56)*
ADENOMA	1 *2%*	0	2 *3.9%*	4 *7.8%*	8** *15.7%*	*2.54% (0-8)*	0	0	1 *2%*	1 *2%*	4* *7.8%*	*2.8% (0-10.2)*
CARCINOMA	0	0	0	4 *7.8%*	9** *17.6%*	*0.47% (0-2.8)*	0	0	0	0	1 *2%*	*0.32% (0-2)*

COMBINED ADENOMA & CARCINOMA	1 *2%*	0	2 *3.9%*	6 *11.8%*	17** *33.3%*	*3.01%* *(0-10)*	0	0	1 *2%*	1 *2%*	5* *9.8%*	*3.12%* *(0-12)*

NB: Level of statistical significance (*p<0.05 and **p<0.01) was only reported for hepatocellular hypertrophy, eosinophilic foci and tumours (in Annex I to CLH report).

Laboratory historical control (HC) data from twenty 104-week dietary studies conducted between 1981 and 2009, ass cited in CLH report and Annex I to CLH report (updated tumour data).

According to the DS, the available evidence suggests that the tumour formation is not a result of genotoxic activity. Hence, a non-genotoxic mode of action (MoA) is plausible. Non-genotoxic modes of actions include cytotoxicity, activation of constitutive androstane receptor (CAR) or peroxisome proliferator-activated receptor alpha (PPARα), porphyria or hormonal perturbation. None of the available data however gave indications of hormonal perturbation, porphyria or increased iron deposition. Since hepatocellular toxicity (e.g. fatty liver and necrosis) has not been observed in any of the rat studies, cytotoxicity is also not a contributing factor. The results of the mechanistic studies further suggest that 1R-trans-Z-momfluorothrin does not act via activation of the aryl hydrocarbon receptor (AhR), the pregnane X receptor (PXR) or PPARα. The latter is supported by the fact that electron microscopy did not provide evidence for peroxisome proliferation. The most plausible MoA is therefore that 1R-trans-Z-momfluorothrin acts via activation of CAR, resulting in altered gene expression specific to CAR activation and subsequently increased cell proliferation and formation of altered hepatic foci.

The postulated MoA involves the following sequence of key events:

1. Nuclear membrane receptor activation (CAR)
2. Altered gene expression specific to CAR activation (e.g. CYP2B)
3. Cell proliferation
4. Clonal expansion to generate altered liver cell foci
5. Increased liver adenoma/carcinoma

Associative events to these key events include CYP2B enzyme induction, liver hypertrophy (including weight), decreased apoptosis, altered epigenetic changes and inhibition of gap junction communication.

In rats, evidence for all key events and for 2 out of 5 associative events (i.e. CYP2B induction and hypertrophy, including increased liver weights) has been provided for 1R-trans-Z-momfluorothrin in the battery of *in vitro* and *in vivo* mechanistic studies and the short- and long-term toxicity studies. Dose-response and time concordance has also been shown. A study with human hepatocytes also demonstrated activation of human hepatic CAR following exposure to 1R-trans-Z-momfluorothrin, but in contrast to rat hepatocytes, no effect of 1R-trans-Z-momfluorothrin on replicative DNA synthesis was seen.

The DS noticed some datagaps and uncertainties. No evidence was provided for decreased apoptosis, inhibition of gap junction intercellular communication or altered epigenetic changes. However, it was considered more important that all key events had been demonstrated. Further, the species differences have not been fully addressed. Indeed, mechanistic studies in mice are consistent with CAR activation but in contrast to rats, 1R-trans-Z-momfluorothrin treatment did not result in increased tumour formation in mice. Yet, this appears to be consistent with findings for other pyrethroids, in particular metofluthrin, which is a close structural analogue to 1R-trans-Z-momfluorothrin.

Despite the above, the DS considers CAR activation the most plausible mechanism behind the liver tumour formation in the rat, based on all the available data. As to the relevance to humans of this MoA, the DS concluded that only certain elements of this MoA are predicted to occur in humans (see Table below). Notably, in human hepatocytes, induction of human CAR was not followed by DNA replication, which is a prerequisite for tumour formation. Hence, the findings with 1R-trans-Z-momfluorothrin were considered consistent with the conclusion expressed in the recent detailed review of Elcombe *et al.* (2014) that this MoA, for which phenobarbital (PB) is the prototypical CAR

activator, is qualitatively not plausible for humans. Consequently, the DS proposed no classification for carcinogenicity as the MoA of tumour formation has very limited or no relevance to humans.

Table Concordance table of Key and associative events for the CAR-mediated MoA, comparing evidence in rats and humans

Key and associative events	**Evidence in rats**	**Evidence in humans**
Activation of CAR	Suggested through the *in vitro* study with RNAi for CAR and induction of CYP2B enzymes	Probable at high doses based on the induction of CYP2B *in vitro*
Altered gene expression	Only changes in CYP2B reported	No experimental evidence in this submission but does occur in other published studies
Induction of CYP2B as a marker for CAR activation	Experimental evidence *in vivo* and *in vitro* with cultured rat hepatocytes	Experimental evidence based on the induction of CYP2B *in vitro*
Hypertrophy	Experimental evidence *in vivo*	Possible based on published evidence in humans treated with anticonvulsant drugs
Increased hepatocellular proliferation	Experimental evidence *in vivo* and *in vitro* with cultured rat hepatocytes	Not reported in cultured hepatocytes
Altered hepatic foci	Experimental evidence *in vivo*	No experimental evidence reported
Liver tumours	Yes	No experimental evidence reported

Comments received during public consultation

One MSCA agreed in general terms on the proposed classification for human health hazards. Another MSCA proposed to classify 1R-trans-Z-momfluorothrin as Carc. 2 because there are clear dose-related increases in hepatocellular adenoma and carcinoma in male and female rats and there is insufficient evidence to rule out the human relevance of the MoA. The MSCA stated that in the key experiment investigating the stimulation of replicative DNA synthesis *in vitro,* the conclusion on human non-relevance was not convincing as stimulation of replicative DNA synthesis was also not clearly demonstrated in rat hepatocytes (inhibitory effect from 100 µM onwards, effect of phenobarbital on increases on replicative DNA synthesis also not convincing). The results observed *in vivo* were accoding to the MSCA not supported by the results observed in this *in vitro* experiment. In response, the DS indicated that based on all available data, classification for carcinogenicity is not required.

Assessment and comparison with the classification criteria

There are no data on long-term exposure and carcinogencity of 1R-trans-Z-momfluorothrin in humans. In animal experiments (a 104-week study in Wistar rats and a 78-week study in CD-1 mice), administration of 1R-trans-Z-momfluorothrin via the diet resulted in increased incidences of liver tumours in rats at 1500 ppm (males) and 3000 ppm (both sexes). 1R-trans-Z-momfluorothrin was not carcinogenic in mice. The mechanism behind the carcinogenicity and the human relevance of the observed liver tumours were investigated/evaluated in several mechanistic studies and a HRF analysis.

In the long-term mouse study a satellite group (12/sex/dose) was exposed for 52 weeks and a main group (52/sex/dose) for 78 weeks. The animals received dietary doses of 0, 600, 2500 or 5500 ppm 1R-trans-Z-momfluorothrin (equal to 0, 72, 308 or 639 mg/kg bw/day for males and 0, 99, 427 or 853 mg/kg bw/day for females). No significant increases in mortality were observed in any dose group, nor any treatment-related tumours. In both the satellite and the main groups, reduced body weight and body weight gain were observed in males at 2500 and 5500 ppm. Liver weight was increased in both groups, in both sexes, mainly at 2500 and 5500 ppm. In the main group this was accompanied by enhanced hepatocellular hypertrophy (from 2500 ppm) and, at the highest dose of 5500 ppm, enhanced single cell necrosis and brownish liver pigmentation. In the satellite group also an increase in hepatocellular hypertrophy was seen, but only in females. Based on these results, 1R-trans-Z-momfluorothrin is considered not to be oncogenic in mice.

In the long-term rat study, animals (51/sex/group) were dosed orally with 0, 200, 500, 1500 or 3000 ppm 1R-trans-Z-momfluorothrin in the diet for 104 weeks (equal to 0, 9.5, 23, 73 or 154 mg/kg bw/day for males and 0, 11, 28, 88 or 182 mg/kg bw/day for females). Survival rate was somewhat lower in females than in males, but was > 50% in all groups. Treatment with 3000 ppm resulted in the premature death of 3 male rats, which was due to the presence of liver tumours. In the 3000 ppm group, liver nodules (13/51 males) and hepatic cysts (6/51 males and 13/51 females) were observed at necropsy. Furthermore, increased incidences of eosinophilic cell foci (20/51 males and 9/51 females), adenoma (8/51 males, 4/51 females), carcinoma (9/51 males, 1/51 females) and combined adenoma and carcinoma (17/51 males, 5/51 females) were observed at this dose. In males at 1500 ppm the incidences of adenoma (4/51), carcinoma (4/51) and combined adenoma and carcinoma (6/51) were also increased, but this was not statistically significant.

Non-neoplastic findings in this study included decreased body weight and body weight gain at 1500 and 3000 ppm, increased relative liver weight at 1500 (22.5% for males and 20.3% for females) and 3000 ppm (65.6% for males and 46.1% for females) and hypertrophy, biliary cysts, cystic degradation and brown pigment in the liver (see Table 2). Brown pigment was also observed in the kidneys at 1500 (31/51 females) and 3000 ppm (25/51 males and 40/51 females). Additionally, diffuse acinar hypertrophy in the mandibular glands was observed in 34/51 males and 24/51 females at 3000 ppm, and thyroid follicular cell hypertrophy in 6/50 males at 3000 ppm (not statistically significant).

Based on the above, 1R-trans-Z-momfluorothrin is considered carcinogenic in rats, with the effect being more marked in male rats than in female rats. For male rats, the incidences of adenomas, carcinomas, and combined adenomas and carcinomas were all at or above the maximum incidence of the historical controls. For female rats, the incidences of adenoma and combined adenoma and carcinoma were within the historical control range, whereas the incidence of carcinoma was equivalent to the maximum incidence of the historical controls.

Mechanistic studies

Several *in vitro* and *in vivo* studies have been conducted to address the MoA responsible for the liver tumour formation. These studies are briefly summarised below (for more details see section 4.10.3 of Background Document and Annex I to Background Document). In several studies, the prototypical CAR activator phenobarbital (PB) was included as a positive control.

In vitro studies with rat hepatocytes

Primary rat hepatocytes (male, Wistar) transfected with CAR siRNA (short interfering RNA specific to CAR, used to block transcription of CAR mRNA and to decrease the amount of functional CAR) or control siRNA (negative control) were exposed to 100 µM 1R-trans-Z-momfluorothrin or to 50 or 500 µM PB. Treatment with CAR siRNA significantly reduced the levels of CAR mRNA in the presence of either 1R-trans-Z-momfluorothrin or PB (to 18-21% of negative controls), which in turn resulted in a significantly reduced induction of CYP2B1/2 mRNA levels by each compound (to 32% and 10-33% of controls, respectively). This indicates that CAR activation is involved in the

induction of CYP2B1/2 mRNA by 1R-trans-Z-momfluorothrin.

In a second study, primary rat hepatocytes (male, Wistar) were treated with 1R-trans-Z-momfluorothrin (1–1000 µM) and PB (500 or 1000 µM) to study CYP2B1/2 induction (by mRNA analysis) and cell proliferation (measured as replicative DNA synthesis). For the latter experiment, also hepatocyte growth factor (HGF; 10 or 100 ng/mL) was tested as a positive control. PB induced CYP2B1/2 by 34- (500 µM) to 45-fold (1000 µM), whereas 1R-trans-Z-momfluorothrin induced CYP2B1/2 by 3-fold at 50 µM, but less at higher concentrations. An increase in DNA replication occurred with PB at both concentrations (maximally 1.4-fold), and with 5 or 10 µM 1R-trans-Z-momfluorothrin (1.6- and 1.8-fold). Remarkably, at higher concentrations of 1R-trans-Z-momfluorothrin (≥ 100 µM), a decrease in DNA replication was observed. Such a decrease was not seen *in vivo* (see below). HGF showed a significant concentration-dependent increase in DNA synthesis (up to 4-fold).

In vitro studies with human hepatocytes
The same type of experiment as described above was performed for human hepatocytes. Primary hepatocytes from up to a total of 10 donors (5 males and 5 females, aged 10 months to 80 years) were treated with 1R-trans-Z-momfluorothrin (1–1000 µM) and PB (1000 µM) to study induction of CYP2B6 (the orthologue to rat CYP2B1/2). Cell proliferation was studied upon treatment with 1R-trans-Z-momfluorothrin (1–1000 µM), PB (500 or 1000 µM) and HGF (10 or 100 ng/mL).
PB treatment led to a 4.8-fold increase in CYP2B6 mRNA, which is much less than the induction of CYP2B1/2 in rat hepatocytes. With 1R-trans-Z-momfluorothrin, 1.7- and 1.8-fold increased levels of CYP2B6 mRNA were seen at 100 and 500 µM, respectively, whereas smaller increases were seen at 50 and 1000 µM. For 1R-trans-Z-momfluorothrin this induction rate is comparable to the one observed in rat hepatocytes. Replicative DNA synthesis was not increased upon treatment with either 1R-trans-Z-momfluorothrin or PB. In fact, decreased replicative DNA synthesis was observed at 100–1000 µM 1R-trans-Z-momfluorothrin, similar to that in rat hepatocytes. With 10 and 100 ng/mL HGF, there was an increase in DNA synthesis by 1.6- and 3.7-fold, showing that the human hepatocytes were capable of a proliferative response.

In vivo rat studies
As the 104-week rat study resulted in increased incidences of adenoma and carcinoma of the liver at 1500 and 3000 ppm, these doses and higher doses of 6000 and 10000 ppm were included in MoA studies with rats. Duration of oral exposure was for 7 or 14 days, and in one study recovery was studied (7-day treatment followed by 7 days of recovery). Investigations included liver weight, CYP2B and CYP4A activity, CYP mRNA levels indicative of AhR, PPARα, PXR and CAR activation, hypertrophy, cell proliferation and electron microscopy. For comparison, male rats were also dosed with 1000 ppm PB in the diet for 7 days.

Treatment-related increases were observed in liver weight, hypertrophy, cell proliferation and CYP2B activity in male and female rats, starting from 1500 ppm (but at 1500 ppm only relatively small effects). These increases were dose-related, and generally more marked after 14 days of treatment than after 7 days. For cell proliferation however the reverse was observed, with higher levels after 7 days of treatment in males (maximally 5.3-fold at 3000 ppm and 17.2-fold at 6000 ppm) and females (2.8-fold at 3000 ppm) as compared to 14 days of treatment (maximally 2.5-fold at 3000 ppm and 4.5-fold at 6000 ppm in males and 1.7-fold at 3000 ppm in females). The effects returned to control levels following a recovery phase of 7 days. 1R-trans-Z-momfluorothrin did not induce CYP1A2, CYP3A1 and CYP3A2 mRNA levels at 3000 ppm. CYP4A activity and CYP4A1 mRNA levels were slightly, but not statistically significantly, increased at 3000 ppm, but electron microscopy did not show evidence of peroxisome proliferation. CYP2B1/2 mRNA levels were markedly increased at 3000 ppm (17.8- and 16.4-fold after 7 and 14 days, respectively). Electron microscopy, however, revealed no increase in SER. PB at 1000 ppm similarly increased CYP2B activity (13-fold), liver weight, hypertrophy and cell proliferation (3.9-fold).

In some of the MoA studies, the effect of 1R-trans-Z-momfluorothrin on the thyroid was investigated, given the increase in thyroid follicular cell hypertrophy in the 52- and 104-week rat studies. When given to rats at 3000, 6000 or 10000 ppm for 7 or 14 days, 1R-trans-Z-momfluorothrin increased the incidence of follicular cell hypertrophy, but did not affect thyroid weight. Slight increases were also seen in hepatic UDP-glucuronosyltransferase (UGT) activity and TSH levels, whereas serum T4 (but not T3) levels were slightly but statistically significantly decreased. These effects suggest an effect on the hypothalamus-pituitary axis similar to PB, for which it is known that induction of hepatic UGT is mediated by CAR. It is therefore likely that the effects of 1R-trans-Z-momfluorothrin on the thyroid are secondary to the liver effects.

In vivo mouse studies
Similar studies as described above were conducted in mice (both sexes), with dietary treatment up to 5500 ppm 1R-trans-Z-momfluorothrin for 7 or 14 days, and including a recovery phase of 7 days. In these studies, mice showed dose-related CYP2B induction/CYP2B activity, increased liver weight, hepatocellular hypertrophy and cell proliferation, and upon electron microscopy an increase in SER was observed. There was also a slight increase in CYP4A induction and CYP4A activity, but electron microscopy did not show an accompanying increase in peroxisome proliferation. Following a recovery phase of 7 days the effects had returned to control levels.

Conclusion
From the mutagenicity data it can be concluded that 1R-trans-Z-momfluorothrin is not genotoxic. Hence, a non-genotoxic MoA is plausible. RAC agrees with the DS that of the known non-genotoxic MoAs behind liver tumour formation in rodents, hormonal perturbation/oestrogens/statins, porphyria, increased iron deposition, infections and increased apoptosis can be discounted for 1R-trans-Z-momfluorothrin, based on all data available. Although some signs of cytotoxicity were observed (e.g. brown pigmentation and cystic degeneration), there was no sustained proliferation and no diffuse, multifocal necrosis, so prolonged cytotoxicity can also be ruled out as the main cause of liver carcinogenicity.

The results of the mechanistic studies further suggest that 1R-trans-Z-momfluorothrin does not act via activation of AhR (no induction of CYP1A2 mRNA was seen), PXR (no evidence for CYP3A1 or CYP3A2 mRNA induction) or PPARα (no evidence for peroxisome proliferation upon electron microscopy). A MoA via immunosuppression is also not likely, as 1R-trans-Z-momfluorothrin is not immunotoxic.

RAC agrees with the DS that CAR activation is the most plausible mechanism behind the liver tumour formation in the rat, given the evidence presented for the key events and some of the associative events in this MoA, also with respect to dose-response relation and temporal association. The *in vitro* study with rat hepatocytes in which the CAR gene was knocked down showed that CAR activation is involved in the induction of CYP2B1/2 mRNA by 1R-trans-Z-momfluorothrin (key event 1). The *in vivo* MoA studies in rats consistently showed CYP2B induction, i.e. increased CYP2B1/2 mRNA expression (key event 2, also shown *in vitro* in rat hepatocytes) and increased CYP2B activity (associative event). MoA studies in mice showed the same picture. Electron microscopy further revealed increased SER (in the 7- and 14-day mouse MoA studies at 5500 ppm and in the 13-week rat study at 6000 ppm), which is characteristic of enzyme inducers.

Increased liver weights and increased incidences of hepatocellular hypertrophy (associative event) were observed in all toxicity (short- and long-term) and MoA studies in rats and mice. Evidence for increased cell proliferation (key event 3) was provided in the rat and mouse MoA studies and in an *in vitro* study with rat hepatocytes. Similar to what is known for PB, the stimulation of cell proliferation by 1R-trans-Z-momfluorothrin was transient and not sustained (i.e. effect smaller after 14 days than after 7 days). However, the overall cell proliferation will still be enhanced due to the increase in total number of hepatocytes.

These events ultimately resulted in increased incidences of eosinophilic foci (key event 4) and liver tumours (key event 5) in rats, but not in mice. This seems inconsistent, as generally mice appear more susceptible than rats to liver tumour formation by CAR activators. However, according to Elcombe *et al.* (2014) for some CYP2B enzyme inducers which appear to have a similar MoA for liver tumour formation to PB, such as pyrethrins and metofluthrin, liver tumours have been observed in the rat and not in the mouse. Metofluthrin is a close structural analogue to 1R-trans-Z-momfluorothrin.

RAC notes that evidence has not been presented for all associative events . However, RAC considers the CAR activation as the most plausible mechanism behind the liver tumour formation in the rat, in line with the DS. As to the relevance to humans of this MoA, the *in vitro* study with human hepatocytes has shown that CAR activation is also possible in humans: 1R-trans-Z-momfluorothrin induced expression of CYP2B6 mRNA. However, 1R-trans-Z-momfluorothrin did not induce replicative DNA synthesis in human hepatocytes, in contrast to rat hepatocytes where 1R-trans-Z-momfluorothrin slightly, but statistically significantly increased cell proliferation. PB, the positive control in the study with human hepatocytes, also induced CYP2B6 mRNA expression, but did not induce cell proliferation that was statistically significantly different compared to controls. In rat hepatocytes on the other hand, and in rats *in vivo*, PB induced replicative DNA synthesis, albeit moderately (maximally 1.4-fold and 3.9-fold, respectively).

RAC acknowledges the argumentation of the DS in discussing the concordance between rat and human evidence for the CAR MoA. RAC further acknowledges that similar to PB, the prerequisite for tumour formation, i.e. DNA replication, does not seem to occur with 1R-trans-Z-momfluorothrin in human hepatocytes following induction of human CAR, in contrast to rats. Due to this qualitative difference, the liver tumours as a result of CAR-activation by 1R-trans-Z-momfluorothrin are considered to be of little relevance to humans. This is in line with a recent review on the human relevance of CAR-mediated liver toxicity, for which PB is the example substance (Elcombe et al., 2014). Hence, RAC supports the conclusion of the DS that 1R-trans-Z-momfluorothrin **does not warrant a classification for carcinogenicity** following a comparison with CLP criteria and an in depth weight of evidence analysis (demonstration that the CAR-mediated MoA is present, that other MoA are excluded and that the relevance to humans is limited).

3.12 Toxicity for reproduction

3.12.1 Effects on fertility

Table 25: Summary table of relevant reproductive toxicity studies - Fertility

Method	Dose levels	Observations and remarks (effects of major toxicological significance)	Reference
Oral (dietary) Rat Wistar 24/sex/group OECD 416 Study compliant with GLP	0, 200,500, 1500 ppm Estimated to be: *P animals:* pre-mating:0, 12.6, 32.1, 95.2 mg/ kg bw/d (males) 0, 14.7, 35.5, 106 mg/ kg bw/d (females) pre-mating to post pairing: 0, 11.6, 29.5, 87.6 mg/ kg bw/d (males) gestation period: 0, 13.1, 31.4, 97.8 mg/ kg bw/d (females) lactation period: 0, 28.0, 68.9, 209 mg/ kg bw/d (females) *F1 animals:* pre-mating period : 0, 14.1, 36.6, 113 mg/ kg bw/d (male) 0, 16.1, 41.3, 126 mg/ kg bw/d (female) pre-mating to post pairing: 0, 13.0, 33.6,	No test item related mortality or clinical signs observed during the study. No treatment related developmental malformations or abnormalities observed in any generation. **At 200 ppm** No test item-related effects were noted. **At 500 ppm** ↓Food consumption and relative food consumption in P generation females. ↓Body weight in the F1 generation males. ↑Absolute liver weights and the ratios of the liver weight to terminal body and brain weights in P generation males. ↑Relative food consumption in F1 generation females. ↓Absolute spleen weights and ratios of the spleen weight to terminal body and brain weights in F1 males and females and F2 females. ↓mean pup weights per dam of F1 and F2 offspring. **At 1500 ppm** ↓Food consumption and body weight in P and F1 generations. Relative food consumptions ↓ in P generation and ↑in F1 generation. ↑Absolute liver weights and the ratios of the liver weight to terminal body and brain weights in P and F1 generations; enlarged liver at necropsy in F1 generation. Sexual maturation was delayed in F1. ↓Body weight, absolute thymus weights, ratios of the thymus weight to brain weights, absolute spleen weights and the ratios of the spleen weight to terminal body (except F2 male pups) and brain weights of F1 and F2 pups. Histopathological lesions recorded in P and F1 generations in the liver (hepatocellular hypertrophy) correlating with increased liver weight.	Pal-Kutas (2012)

	104 mg/ kg bw/d (males) gestation: 0, 13.8, 35.4, 108 mg/ kg bw/d (females) lactation: 0, 28.9, 76.0, 247 mg/ kg bw/d (females)		

3.12.1.1 Non-human information

The effects of 1R-trans-Z-momfluorothrin on fertility have been investigated in a multigeneration study in Wistar rats. No effects were seen on mating performance, number of pregnant animals, and number of implantations or post-implantation loss. An increase was seen in the time taken to reach preputial separation or vaginal opening which reached statistical significance in the highest dose group for both sexes. In both sexes the mean body weight at pubertal maturation was reduced. This effect is therefore considered to be secondary to general toxicity.

3.12.1.2 Human information

No data available

3.12.2 Developmental toxicity

Table 26: Summary table of relevant developmental toxicity studies - Development

<table>
<tr><th>Method</th><th>Dose levels</th><th>Observations and remarks

(effects of major toxicological significance)</th><th>Reference</th></tr>
<tr><td>One generation developmental

Oral (gavage)

Rat, (SD)

Female 22-24/group

OECD 414

Study compliant with GLP and OECD guidelines</td><td>0, 10, 25, 75 mg/ kg bw/d in corn oil

Gestation days 6-19

Purity 95.7%</td><td>Maternal toxicity

25 mg/kg bw/d
Slight ↓food consumption sporadically throughout study.

75 mg/kg bw/d
Tremors in 6 animals at a frequency of 1-2 animals per day during late gestation. ↓body weight on day 20 of gestation. Slight ↓food consumption sporadically during study,

Reproductive toxicity
↓number of corpora lutea, ↓number of implantations and ↓number of live foetuses.

<table>
<tr><th>Dose level, mg/kg bw/day</th><th>0</th><th>10</th><th>25</th><th>75</th></tr>
<tr><td>Mean gravid uterine weight (g)</td><td>77.46</td><td>77.25</td><td>75.01</td><td>71.32</td></tr>
<tr><td>No. of corpora lutea/dam</td><td>15.4</td><td>15.1</td><td>14.7</td><td>14.2*</td></tr>
<tr><td>No. of implantations/dam</td><td>14.7</td><td>14.3</td><td>14.3</td><td>13.7</td></tr>
</table>
</td><td>Izumi (2012)</td></tr>
</table>

		<table><tr><td>No of live foetuses/dam</td><td>14.3</td><td>13.6</td><td>13.5</td><td>13.0</td></tr></table> Level of statistical significance: * 0.05> p > 0.01 in comparison with control **Foetal**: No effects at the highest dose level tested.	
One generation developmental Oral (gavage) Rabbit, New Zealand White (Kbl:NZW) Female 24/group OECD 414 Study compliant with GLP and OECD guidelines	0, 100, 300 1000 mg/kg bw/d in 0.5% aqueous methylcellulose Gestation days 6-27 Purity 95.7%	**Maternal toxicity** **300 and 1000 mg/kg bw/d** slight ↓in food consumption during the first 3 days of dosing. **Foetal toxicity:** No effects at the highest dose tested.	Iwashita (2012)

3.12.2.1 Non-human information

The potential for 1R-trans-Z-momfluorothrin to cause developmental toxicity has been investigated in rats and rabbits in two developmental toxicity studies and one multigeneration study in rats (see Section 4.11.1).

Rats

1R-trans-Z-momfluorothrin (as S-1563) was administered by gavage to groups of 22 to 24 female Crl:CD SD rats from days 6 to 19 of gestation to investigate the effects on dams and embryo-foetal development (Izumi, 2012). Animals were exposed to S-1563 at 10, 25 or 75 mg/kg bw/d. No deaths occurred in any of the groups. There was no effect on food consumption or necropsy findings. The changes in the number of corpora lutea, gravid uterine weight, number of implantations and live foetuses is considered non-treatment related as these parameter were established prior to the start of dosing. At the top dose level, tremor was observed in 6 animals at a frequency of 1-2 animals per day during late gestation. These are considered to be due to treatment with S-1563.

Caesarean section data showed no effect on embryo-foetal development. Similarly in the multigeneration study, no foetal malformations or abnormalities were reported up to the top dose.

Rabbits

1R-trans-Z-momfluorothrin as S-1563 was administered by gavage to groups of 24 female New Zealand White (Kbl:NZW) from days 6 to 27 post-insemination (Iwashita, 2011). Rabbits were dosed with test substance at 100, 300 or 1000 mg/kg bw/d. No maternal deaths occurred in any of the treatment groups and no treatment related clinical effects were seen in dams. Food consumption was significantly reduced in animals dosed at 300 and 1000 mg/kg bw/d during the first 3 days of

dosing. There was no other evidence of maternal toxicity. No evidence of an effect on embryo-foetal development was reported.

3.12.2.2 Human information

No data available.

3.12.3 Other relevant information

None available.

3.12.4 Summary and discussion of reproductive toxicity

The potential of 1R-trans-Z-momfluorothrin to cause reproductive toxicity has been investigated in two teratology studies in rats and rabbits and one multigeneration study in rats.

Fertility
No effects were seen on fertility.

Development
No effects were seen on developmental parameters (except the time taken to reach sexual maturity, which is considered secondary to general toxicity (the retarded growth of the pups)). No foetal malformations or abnormalities of concern were noted in any of the studies.

3.12.5 Comparison with criteria

Fertility
No effects were observed that provide evidence to suggest that 1R-trans-Z-momfluorothrin adversely affects sexual function or fertility in the absence of systemic toxicity.

Development
No effects were observed that provide evidence to suggest that 1R-trans-Z-momfluorothrin adversely affects development.

3.12.6 Conclusions on classification and labelling

No classification required

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

The potential of 1R-trans-Z-momfluorothrin to affect reproduction was assessed using three GLP compliant studies, one in rats conducted according to OECD TG 416 (two-generation reproduction toxicity study), and two studies conducted according to OECD TG 414 (prenatal development toxicity study), one in rats and one in rabbits.

In the two-generation reproduction toxicity study (Pal-Kutas, 2012), rats received 0, 200, 500 or 1500 ppm 1R-trans-Z-momfluorothrin in the diet. No mortalities or clinical signs were observed and there were no effects on mating performance, the number of pregnant animals, and the number of implantations or post-implantation loss. Main effects in the

parental generations at 1500 ppm included reduced food consumption and body weight (up to 10%) and increased liver weight (up to 30%) with correlating histopathological findings (hepatocellular hypertrophy) in both sexes. Body weight and liver weight were also slightly affected at 500 ppm in F1 and P generation males, respectively, and food consumption was reduced at 500 ppm in P generation females. In the offspring, pup weights up to weaning were decreased at 500 (<10%) and 1500 ppm (10-16%) in F1 and F2 as well as at 200 ppm in F2 (without a clear dose-response in F2). F1 pups showed a statistically significant delay in sexual maturation (26.3 *vs*. 25.0 days for preputial separation, 37.0 *vs.* 34.1 days for vaginal opening) at 1500 ppm, a dose at which the F1 males had 12% decreased body weight at maturation. The DS considered the effects on sexual maturation as secondary to general toxicity. In pups of both generations, reductions were seen in absolute thymus weight at 1500 ppm and in absolute and relative spleen weights at 1500 and 500 ppm, without any histopathological findings.

Developmental toxicity of 1R-trans-Z-momfluorothrin was tested in rats at oral (gavage) levels of 0, 10, 25 and 75 mg/kg bw/day from gestation days 6-19. No maternal deaths occurred during the treatment period. At 75 mg/kg bw/day, tremors were observed in 6 animals at a frequency of 1-2 animals per day during late gestation. The number of corpora lutea was decreased, and the gravid uterine weight, number of implantations and live foetuses were reduced. However, as the number of corpora lutea was established prior to dosing, the effect on this parameter is not treatment-related. As the decrease in the gravid uterine weight of dams and in the number of implantations and live foetuses are a consequence of the lower number of corpora lutea, these effects are also considered not treatment-related. No effects on embryo-foetal development, foetal malformations or abnormalities were seen up to and including the highest tested dose.

Developmental toxicity of 1R-trans-Z-momfluorothrin was also tested in rabbits, at oral (gavage) levels of 0, 100, 300 and 1000 mg/kg bw/day from gestation days 6-27. No maternal deaths occurred during the treatment period. Significant reduction in food consumption was observed during the first 3 days of dosing in the 300 and 1000 mg/kg bw/day groups. In the 300 mg/kg bw/day group, this effect persisted until day 15 of gestation. No other effects were observed in the dams. There were also no effects on embryo-foetal development.

Overall, the DS concluded that 1R-trans-Z-momfluorothrin has no adverse effects on sexual function and fertility or on development and therefore proposed no classification for reproductive toxicity.

Comments received during public consultation

No specific comments were received during PC, but 2 MSCAs agreed in general terms on the proposed classification for human health hazards.

Assessment and comparison with the classification criteria

No effects on reproductive organs have been described for the repeated dose toxicity studies presented in the CLH dossier. In the rat two-generation study, levels up to and including the highest dose of 1500 ppm 1R-trans-Z-momfluorothrin (87.6-126 mg/kg bw/day) did not produce an adverse effect on reproductive performance/parameters. RAC therefore agrees with the DS that there is no need to classify 1R-trans-Z-momfluorothrin for effects on sexual function and fertility.

In the available developmental toxicity studies, 1R-trans-Z-momfluorothrin did not adversely affect development in rats and rabbits. In the rat two-generation study, a delay in sexual maturation was seen at 1500 ppm, but this is probably secondary to some general toxicity observed at this dose and the mid dose of 500 ppm, both in pups (reduction in body weight

and in some organ weights) and in parental animals (reductions in body weight and food consumption, and effects on the liver). In conclusion, RAC agrees with the DS that these effects **do not warrant classification of 1R-trans-Z-momfluorothrin for developmental toxicity**.

3.13 Other effects

3.13.1 Non-human information

3.13.1.1 Neurotoxicity

The neurotoxicity of 1R-trans-Z-momfluorothrin has been investigated in two guideline studies. In an acute neurotoxicity study, rats were dosed with 1R-trans-Z-momfluorothrin (as S-1563) in corn oil. Clinical signs of neurotoxicity included tremors (3/10F), increased levels of salivation (3/10M) and straub tail (1/10M and 1/10F). Refer to section 4.2 for full details. This finding is similar to that observed in the acute oral toxicity test where clinical effects indicating a neurotoxic effect were observed at 300 mg/kg bw and above.

In a second neurotoxicity study animals dosed with up to 402/425 mg/kg/day (M/F) S-1563 (in the diet for 90 days showed no signs of neurotoxicity, although systemic toxicity (reduced body weight gain) was seen (refer to section 4.7).

3.13.1.2 Immunotoxicity

Table 27: Summary of immunotoxicity studies

Method	Dose Levels	Observations and Remarks	Reference
28 day study (oral, dietary) Rat, Wistar Crl: WI(HAN) Oral (dietary) 10 male/group Purity: 95.7% USEPA OPPTS870.7800 Study compliant with GLP and USEPA guidelines	0, 300, 1000, 3000 ppm (0, 26, 81, 241 mg/kg bw/d)	No effects observed on the functional humoral immune response. Reduced body weight (-7.3 %) and lower food consumption in high dose group. Negative control group received basal diet only. Positive control group was treated with 50 mg/kg bw/d of cyclophosphamide monohydrate (CPS) for four days.	Hosako (2011)

The only treatment-related effects seen in animals dosed with S-1563 were lower body weight and reduced food consumption in animals in the 3000 ppm test group. Treatment with S-1563 produced no statistically significant effects on spleen cell number, nor did it significantly suppress the humoral immune response when evaluated as either specific activity (AFC/10^6 spleen cells) or as total activity (AFC/spleen) of splenic IgM AFC response to the T cell-dependent antigen sRBC.

Animals treated with CPS showed the expected reduction in spleen cell numbers and total spleen activities of IgM antibody-forming cells.

3.13.1.3 Specific investigations: other studies

3.13.1.4 Human information

None available.

3.13.2 Summary and discussion

Neurotoxicity
A summary of the neurotoxic effects is provided in section 4.3.2.

Immunotoxicity
No treatment related effects on the functional humoral response were observed at systemically toxic doses.

3.13.3 Comparison with criteria

Neurotoxicity

Comparison with the criteria for classification on the basis of neurotoxic effects has been considered under section 4.3.2.

Immunotoxicity

No criteria are established under CLP for classification for immunotoxic effects. However as no functional humoral immune response was observed in tests and so 1R-trans-Z-momfluorothrin is considered not to be immunotoxic. No classification is proposed.

3.13.4 Conclusions on classification and labelling

Classification with Acute Tox 4; H302 Harmful if swallowed and STOT-SE 2; H371 May cause damage to organs (Central Nervous System) is proposed under sections 4.2 and 4.3

4 ENVIRONMENTAL HAZARD ASSESSMENT

1R-trans-Z-momfluorothrin is an insecticide used as a spray or crack and crevice treatment (indoors and outdoors) to control flying insects (e.g. mosquitoes) and crawling insects (e.g. black ants, cockroaches and bed bugs). Available environmental fate and hazard studies have been reviewed under the Biocidal Products Regulation (BPR), (EU) 528/2012. The studies are summarised in the Competent Authority Report (CAR). The key information pertinent to determining a classification is presented below.

The technical material used for the generation of the majority of the environmental hazard data was referred to by the manufactures code 'S-1563' in the study reports. S-1563 is quoted as having a minimum purity of 95.2% (based on total momfluorothrin isomer content) in the study reports, but it is predominantly the RTZ isomer (>86% RTZ). Some of the fate data have been generated on the pure isomers (i.e. the RTZ or RTE isomers with a purity of >99%), where this is the case it is specified in the dossier.

The substance degrades to a number of degradants in the environment which are identified in Figure 1. Non GLP acute toxicity to algal, *Daphnia* and fish data (Miyamoto *et al*, 2013a, 2013b and 2013c) are available for the degradants MFOA-D, MFOA and *t*-COOH-CA indicating they are significantly less toxic than the parent. This report therefore focuses on the classification of 1R-trans-Z-momfluorothrin alone.

4.1 Degradation

A summary of available information on the fate of 1R-trans-Z-momfluorothrin is presented in Table 28 below.

Table 28: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Aquatic hydrolysis OECD Guideline 111, GLP	Stable at pH 4 DT_{50} at 40°C , pH 7: 56.3- 77.5 days DT_{50} at 25°C , pH 9: 6.5-7.2 days	Valid study	Ponte, 2011a
Aquatic photolysis OECD Guideline 316, GLP	DT_{50} 13.4 days equivalent to 25.9 OECD solar days DT_{50} (including photoisomerisation) 14.5 OECD solar days	Valid study	Ponte, 2012
Ready biodegradation OECD Guideline 301B, GLP	Not rapidly biodegradable -5.46 to 3.91% degradation	Valid study	Ilic, 2010
Water/sediment simulation OECD Guideline 308, GLP	DT_{50} 0.6 to 2.9 days based on parent dissipation whole system Mineralisation: 15% AR* day 100 to 43% AR day 105	Valid study	Ponte, 2011

*AR refers to Applied Radioactivity

4.1.1 Stability

Aqueous hydrolysis

An aqueous hydrolysis study (Ponte, 2011a) is available following GLP and OECD Guideline 111. The study used 3 radio labels (purity ≥ 99%):

- [alc-^{14}C] 1R-trans-Z -momfluorothrin: [methoxymethylbenzyl-α-^{14}C] 1R-trans-Z-momfluorothrin
- [acid-^{14}C] 1R-trans-Z-momfluorothrin: [cyclopropyl-1-^{14}C] 1R-trans-Z-momfluorothrin
  [acid-^{14}C] 1R-trans-E-momfluorothrin: [cyclopropyl-1-^{14}C] 1R-trans-E-momfluorothrin

A preliminary test was run at pH 4, 7 and 9 at 50 ± 0.1^{o}C for up to 5 days with 2 isomers: [acid-^{14}C] 1R-trans-Z-momfluorothrin and [acid-^{14}C] 1R-trans-E-momfluorothrin. The momfluororthrin isomers were considered stable at pH 4 whilst significant hydrolysis was observed at pH 7 and 9. It was noted that hydrolysis of the 2 isomers was not significantly different.

Subsequently a definitive test was conducted at pH 7 (up to 33 days for 40, 50, and 60^{o}C) and pH 9 (up to 21 days for 25, 40 and 50^{o}C) using 2 isomers: [alc-^{14}C] 1R-trans-Z-momfluorothrin and [acid-^{14}C] 1R-trans-Z-momfluorothrin. Radiocarbon recovery was determined by Liquid Scintillation Counting (LSC). The degradation rates at pH 7 and 9 were calculated using pseudo-first order kinetics. Similarly, it was noted that hydrolysis of the 2 isomers was not significantly different.

The DT_{50} values were pH dependant and presented at follows:

- pH 7: 660 to 1394 days at 20^{o}C
- pH 9: 11.7 to 12.2 days at 20^{o}C

Parent and degradants were quantified by High Performance Liquid Chromatography (HPLC) and confirmed by Thin Layer Chromatography (TLC). The study concluded that 1R-trans-Z-momfluorothrin undergoes hydrolytic cleavage of the ester link to form carboxylic acid and alcohol, with degradants Z-CMCA and MFOA. Z-CMCA was the principal degradant of the acid label isomer and MFOA the principal degradant of the alcohol label isomer.

Aqueous Photolysis

An aqueous photolysis study (Ponte, 2012) is available following GLP and OECD Guideline 316.

The study used 3 radio labels (purity ≥ 99%):

- [alc-^{14}C] 1R-trans-Z-momfluorothrin: [methoxymethylbenzyl-α-^{14}C] 1R-trans-Z-momfluorothrin
- [acid-^{14}C] 1R-trans-Z-momfluorothrin: [cyclopropyl-1-^{14}C] 1R-trans-Z-momfluorothrin
- [acid-^{14}C] 1R-trans-E-momfluorothrin: [cyclopropyl-1-^{14}C] 1R-trans-E-momfluorothrin

The study was run at pH 4 with solutions exposed to continuous artificial light (Xenon arc lamp average light intensity of 443 W/m^2 for the 300-800nm range) considered equivalent to natural midsummer sunlight at 37.45^{o}N latitude, for 13 days at 25^{o}C ± 2^{o}C. Under light conditions, extensive isomerisation was observed with no significant differences between the RTZ and RTE label. However, none of the other 7 isomers were >4.7% AR.

Radiocarbon recovery was determined by LSC. Parent and degradants were quantified by HPLC.

The Quantum Yield (calculated as the sum of 8 momfluorothrin isomers) was 0.0113 and including photoisomerisation 0.0205.

The mean DT_{50} was 13.4 days under artificial light equating to 25.9 OECD solar days. Including photoisomerisation, the mean DT_{50} was 7.5 days under artificial light equating to 14.5 OECD solar days.

The main degradants were identified as CMCA and MFOA via cleavage of the ester link. CMCA was the principal degradant of the acid label isomer and MFOA the principal degradant of the alcohol label isomer

4.1.2 Biodegradation

4.1.2.1 Biodegradation estimation

None available.

4.1.2.2 Screening tests

A ready biodegradation study (Ilic, 2010) is available following OECD Guideline 301B (CO_2 Evolution) and GLP using S-1563 (purity 95.6%, total momfluorothrin isomer content). Test solutions were prepared with 100 mg test item in ~3 litres meaning the substance was tested above the water solubility of 0.6 to 1.4 mg/l. The study was run a pH 7.53 to 7.68 and between 21 and 24^{o}C. Validation criteria for the Reference and Toxicity Controls were met. Ultimate biodegradation reached a maximum of 3.91 %. Overall, the substance is considered not readily biodegradable.

4.1.2.3 Simulation tests

A degradation in water-sediment system study (Ponte and Mannella, 2011) is available following OECD Guideline 308 and GLP. Two aerobic systems were used: Calwich, Derbyshire referred to as ‘Abbey’ and Chatsworth, Derbyshire referred to as ‘Swiss’. The test conditions are included in table 29 below.

Table 29: Water-sediment system test conditions

Criteria	Abbey	Swiss
Sediment properties	33% sand; 56% silt; 11% clay Organic matter 8% pH in 1:1 sediment: water ratio: 7.6	95 % sand; 4% silt; 1% clay Organic matter 1.4% pH in 1:1 sediment: water ratio: 6.1
Water properties	pH: 8.1 Hardness: 233 mg equivalent $CaCO_3$/L Total dissolved solids: 304 ppm Total suspended solids: 16 ppm	pH: 7.1 Hardness: 22 mg equivalent $CaCO_3$/L Total dissolved solids: 72 ppm Total suspended solids: 14 ppm
Characteristics and maintenance of the test system during study	Oxidation reduction potential in water layer: 69 mV in sediment: -45 mV pH in water layer: 7.36 in sediment: 7.31 dissolved oxygen: 4.56 ppm	Oxidation reduction potential in water layer: 145 mV in sediment: 185 mV pH in water layer: 7.00 in sediment: 6.59 dissolved oxygen: 6.57 ppm

The study used 3 radio labels (>99% purity):

- [alc-^{14}C] 1R-trans-Z-momfluorothrin: [Methoxymethylbenzyl-α-^{14}C] 1R-trans-Z-momfluorothrin
- [acid-^{14}C] 1R-trans-Z-momfluorothrin: [Cyclopropyl-1-^{14}C] 1R-trans-Z-momfluorothrin
- [acid-^{14}C] 1R-trans-E-momfluorothrin: [Cyclopropyl-1-^{14}C] 1R-trans-E-momfluorothrin

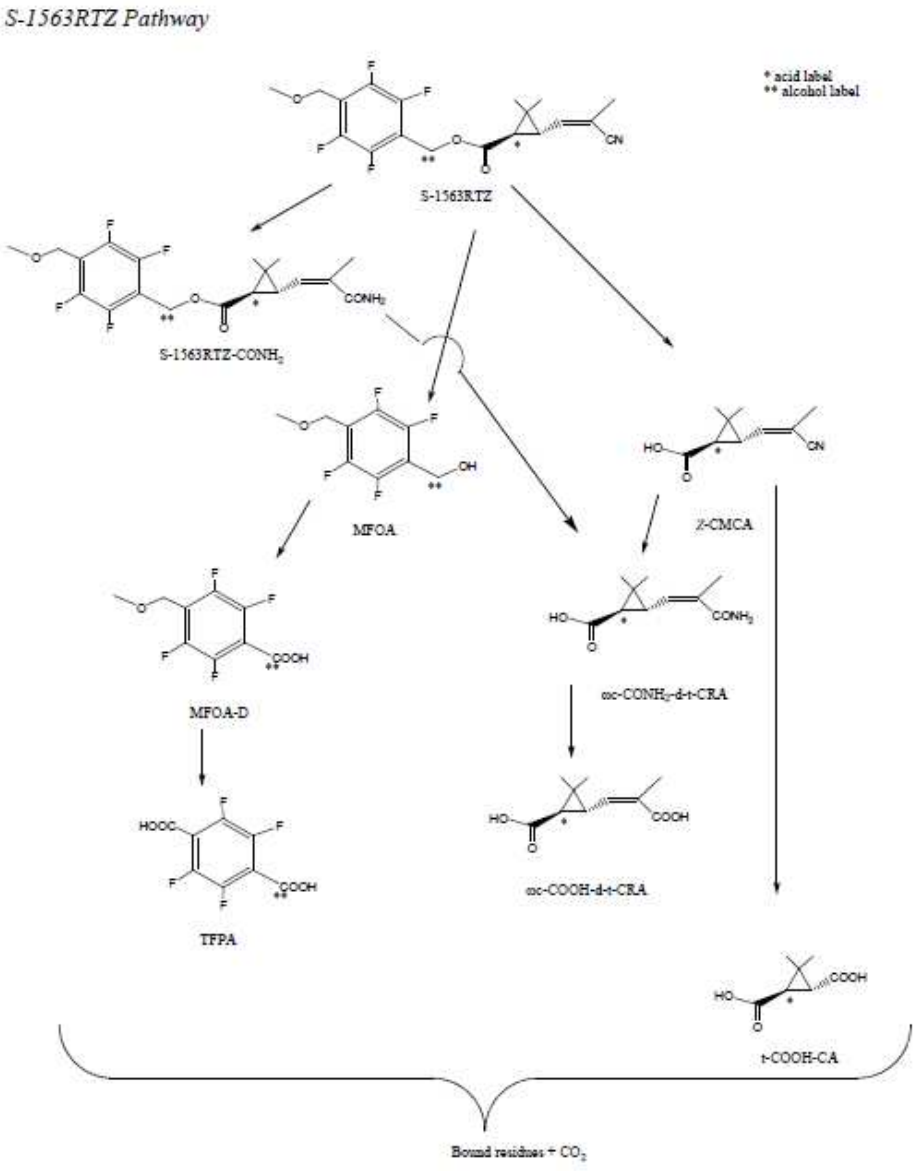
Initial nominal dose rates were 7.7 to 8.4 µg per sample with direct addition to the water phase. The study was run in the dark at 20 ± 2°C for between 100 and 146 days depending on the radio label. The water layers and sediment extracts were quantified by LSC. Following extraction, the residual radioactivity in sediment was determined by combustion and radioassay. 1R-trans-Z/E - momfluorothrin and degradants were quantified by HPLC. No significant further isomerisation was observed in any of the test systems.

Overall, 1R-trans-Z –momfluorothrin and 1R-trans-E-momfluorothrin were observed to rapidly dissipate from the water column in both systems representing <4% Applied Radioactivity (AR) after 8 days. Identified degradants are presented in Table 30. Figure 1 shows the proposed degradation pathway. Table 31 presents the distribution of parent and degradants.

Table 30: Identified degradants in aerobic water-sediment system

Radiolabel and isomer	Degradants
[alc^{14}C] 1R-trans-Z-momfluorothrin	Major degradants: MFOA (depending on test system), MFOA-D and TFPA
[acid^{14}C] 1R-trans-Z-momfluorothrin	Major degradant: Z-CMCA Minor degradants: ωc-$CONH_2$-d-t-CRA and t-COOH-CA (depending on test system)
[acid^{14}C]1R-trans-E-momfluorothrin	Major degradants: *E*-CMCA, ωt-$CONH_2$-d-t-CRA and t-COOH-CA (depending on test system)

Figure 1: Proposed degradation pathway of 1R-trans-Z-momfluorothrin (S-1563 RTZ) and 1R-trans-E-momfluorothrin (S-1563 RTE) in water-sediment systems under aerobic conditions



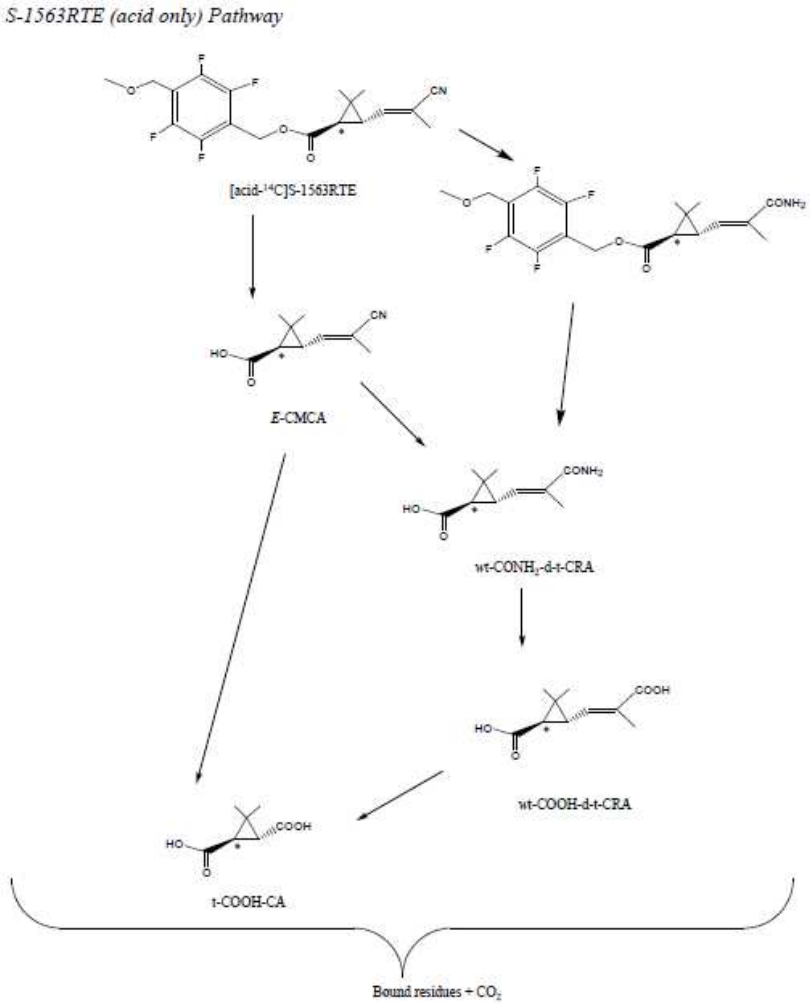


Table 31: Distribution of 1R-trans-Z-momfluorothrin (S1563 RTZ) and 1R-trans-E-momfluorothrin (S-1563 RTE) and degradants as % Applied Radioactivity in aerobic water-sediment systems

[alc-^{14}C]S-1563RTZ									
Abbey Test System									
Time (days)	S-1563RTZ	S-1563RTE	MFOA	S-1563RTZ-$CONH_2$	MFOA-D	TFPA	Bound Residues	Organic Volatiles	(CO_2)
0	88.6	3.0	0.7	0.0	1.1	0.0	1.9	NA	NA
1	30.4	0.0	19.3	3.7	39.4	1.2	5.0	0.0	0.6
3	14.6	0.0	23.1	2.5	47.5	1.0	0.7	0.1	0.0
8	2.9	0.7	35.0	0.0	49.3	0.2	1.1	0.0	0.2
15	1.5	0.5	24.9	0.4	61.7	0.5	1.6	0.0	0.1
31	0.3	0.5	23.0	0.0	59.7	4.6	2.6	0.0	0.6
63	0.7	0.0	7.0	0.0	68.1	6.0	3.1	0.1	2.6
100	0.5	0.0	0.3	0.0	72.9	10.5	3.0	0.1	6.6
146	0.0	0.0	0.8	0.2	64.1	5.3	4.8	0.3	18.7
Swiss Test System									

Time (days)	S-1563RTZ	S-1563RTE	MFOA	S-1563RTZ-$CONH_2$	MFOA-D	TFPA	Bound Residues	Organic Volatiles	(CO_2)
0	90.1	2.9	1.4	0.0	0.0	0.0	0.7	NA	NA
1	65.2	2.5	5.9	3.0	9.9	0.6	1.6	0.0	0.3
3	39.0	0.2	9.5	7.3	34.5	1.5	0.5	0.0	0.1
8	3.0	0.3	0.0	4.5	73.2	10.1	0.4	0.0	0.3
15	0.9	0.2	0.9	1.9	74.5	11.4	0.4	0.1	0.4
31	1.1	0.2	0.8	2.1	74.0	10.0	0.6	0.0	1.1
63	0.7	0.0	0.3	0.2	71.4	19.0	0.5	0.1	2.5
100	0.4	0.0	0.0	0.0	58.9	32.4	0.8	0.0	0.9
146	0.2	0.0	0.0	0.0	11.1	67.5	1.8	0.2	19.4

[acid-^{14}C]S-1563RTZ

Abbey Test System

Time (days)	S-1563RTZ	S-1563RTE	Z-CMCA	S-1563RTZ-$CONH_2$	wc-$CONH_2$-d-t-CRA	t-COOH-CA	wc-COOH-d-t-CRA	Bound Residues	Organic Volatiles	(CO_2)
0	85.9	4.2	1.4	0.0	0.0	0.0	0.0	1.3	NA	NA
1	46.0	2.1	39.6	2.3	1.1	0.0	0.0	3.8	0.0	0.1
3	14.9	1.4	69.6	3.0	2.4	0.0	0.0	2.2	0.1	0.9
8	1.4	0.7	83.7	0.0	3.6	0.0	0.0	1.9	0.1	1.0
13	0.9	0.5	78.1	0.0	5.5	1.1	1.3	3.0	0.0	2.7
30	0.6	0.0	71.6	0.0	7.4	3.1	1.0	5.0	0.0	3.3
58	0.5	0.0	37.0	0.0	6.0	10.3	0.0	16.5	0.5	15.4
105	0.0	0.0	4.1	0.0	2.3	4.2	0.0	27.2	0.3	43.0

Swiss Test System

Time (days)	S-1563RTZ	S-1563RTE	Z-CMCA	S-1563RTZ-$CONH_2$	wc-$CONH_2$-d-t-CRA	t-COOH-CA	wc-COOH-d-t-CRA	Bound Residues	Organic Volatiles	(CO_2)
0	87.9	4.0	1.9	0.0	0.0	0.0	0.0	0.5	NA	NA
1	54.2	1.9	23.6	4.5	1.0	1.6	0.5	3.0	0.0	0.3
3	20.5	1.3	57.8	7.3	1.7	2.4	0.0	2.9	0.0	2.5
8	1.6	0.6	72.0	3.8	2.8	2.4	0.0	5.7	0.0	3.7
13	0.8	0.3	69.0	2.8	4.0	2.9	0.0	7.1	0.0	4.9
30	0.0	0.0	62.4	0.2	5.6	4.8	0.7	7.8	0.1	10.3
58	0.2	0.0	25.2	0.0	10.6	4.7	1.0	14.4	0.1	34.2
100	0.0	0.0	20.4	0.0	21.0	6.1	0.0	14.4	0.4	18.0

[acid-^{14}C]S-1563RTE

Abbey Test System

Time (days)	S-1563RTRE	S-1563RTZ	E-CMCA	S-1563RTE-$CONH_2$	wt-$CONH_2$-d-t-CRA	t-COOH-CA	wt-COOH-d-t-CRA	Bound Residues	Organic Volatiles	CO_2
0	90.8	3.2	0.8	0.0	0.0	0.0	0.0	1.8	NA	2.1
1	53.5	2.3	29.0	4.3	1.7	1.8	0.0	4.7	0.5	0.3
3	15.8	0.7	62.1	4.2	4.2	4.1	0.1	1.9	0.1	0.6
7	1.3	1.5	64.3	2.6	8.0	8.5	1.2	4.0	0.0	1.6
14	1.4	0.0	58.8	0.8	11.5	9.2	0.6	4.8	0.0	3.1
30	0.8	0.3	53.2	0.1	12.1	11.7	0.7	7.3	2.1	5.0
59	0.5	0.0	22.9	0.1	20.1	21.5	0.6	14.3	0.4	14.2
100	0.5	0.0	2.6	0.0	9.4	6.9	0.3	28.5	0.9	38.2

Swiss Test System

Time (days)	S-1563RTRE	S-1563RTZ	E-CMCA	S-1563RTE-$CONH_2$	wt-$CONH_2$-d-t-CRA	t-COOH-CA	wt-COOH-d-t-CRA	Bound Residues	Organic Volatiles	CO_2
0	91.0	3.7	1.4	0.0	0.0	0.0	0.0	0.6	NA	3.3
1	54.8	2.8	22.2	7.3	5.8	1.5	0.6	2.6	0.0	1.0
3	22.7	0.9	43.5	7.2	11.4	4.2	2.7	1.4	0.0	1.5
7	2.4	1.6	54.8	4.0	16.7	6.3	4.1	1.9	0.0	0.4
14	1.0	0.0	51.4	3.1	13.8	13.7	1.8	4.9	0.1	4.5
30	0.7	0.3	59.8	0.8	9.9	10.8	2.4	3.5	0.0	5.7
59	1.0	0.1	9.9	1.4	12.9	23.3	0.8	12.2	0.1	27.4
100	0.2	0.0	7.4	0.7	31.7	26.1	3.8	7.9	0.0	15.0

In addition to the formation of degradants, mineralisation was observed and this is presented in Table 32.

Table 32: Mineralisation as % Applied Radioactivity in aerobic water-sediment systems

Radiolabel	Swiss	Abbey
[alc^{14}C] 1R-trans-Z-momfluorothrin	19.4 % AR day 146	18.7 % AR day 146
[acid^{14}C] 1R-trans-Z-momfluorothrin	34.2 % AR day 58	43 % AR day 105
[acid^{14}C] 1R-trans-E-momfluorothrin	15 % AR day 100	38.2 % AR day 100

Degradation rates (DT_{50} and DT_{90}) for 1R-trans-Z-momfluorothrin and 1R-trans-E-momfluorothrin and the degradants were calculated using Single First Order (SFO) model, Double First Order Parallel (DFOP) model and First Multi Component Order (FOMC) using KinGui version 1.1 software. The values are presented in Table 33.

Table 33: DT_{50} half-lives and DT_{90} values for 1R-trans-Z-momfluorothrin and 1R-trans-E-momfluorothrin and its degradants in aerobic water-sediment systems

[alc-^{14}C] 1R-trans-Z-momfluorothrin								
Test System		Parent		MFOA		MFOA-D		
		Abbey	Swiss	Abbey	Swiss	Abbey	Swiss	
Whole system	DT_{50}	0.6	2.9	35.8	MFOA was not considered for calculation as it represented < 10% AR	>1000	103.2	
	DT_{90}	4.8	9.7	118.8		>1000	342.9	
	Model	FOMC	SFO	SFO		SFO	SFO	
Water layer	DT_{50}	0.1	2.5	26.3		>1000	107.9	
	DT_{90}	2.0	8.2	87.4		>1000	358.4	
	Model	DFOP	SFO	SFO		SFO		

[acid-^{14}C] 1R-trans-Z-momfluorothrin					
Test System		Parent		Z-CMCA	
		Abbey	Swiss	Abbey	Swiss
Whole system	DT_{50}	1.2	1.6	43.5	44.8
	DT_{90}	3.9	5.4	144.5	148.8
	Model	SFO			
Water layer	DT_{50}	1.0	1.4	30.4	41.7
	DT_{90}	3.3	4.7	101.1	138.6
	Model	SFO			

[acid-^{14}C]1R-trans-E-momfluorothrin									
Test System		Parent		*E*-CMCA		ωt-CONH$_2$-d-t-CRA		t-COOH-CA	
		Abbey	Swiss	Abbey	Swiss	Abbey	Swiss	Abbey	Swiss
Whole system	DT_{50}	1.3	1.6	33.6	36.5	>1000	*	>1000	*
	DT_{90}	4.4	5.4	111.7	121.1	>1000		>1000	
	Model	SFO							
Water layer	DT_{50}	1.0	1.4	25.4	36.1	>1000	*	>1000	*
	DT_{90}	3.2	4.5	84.5	120.1	>1000		>1000	
	Model	SFO							

*Degradation rates were not determined for ω t-CONH$_2$-d-t-CRA and t-COOH-CA as concentrations increased until the end of the study.

4.1.3 Summary and discussion of degradation

1R-trans-Z-momfluorothrin and 1R-trans-E-momfluorothrin are considered to degrade similarly with no significant difference anticipated.

1R-trans-Z-momfluorothrin is considered stable under acidic conditions with limited hydrolysis at neutral pH. Under alkaline conditions, 1R-trans-Z-momfluorothrin undergoes hydrolysis with an experimental half-life of 6.5 to 7.2 days at 25^{o}C. Converting this half-life to a more environmentally relevant temperature of 12^{o}C results in values of 18.3 to 20.3 days (equation from the REACH *Guidance on information requirements and chemical safety assessment, Chapter R.7b*[1]).

Overall, 1R-trans-Z-momfluorothrin is considered stable over the majority of the environmentally relevant pH range. At higher pH, abiotic degradation is significant although it is likely to reflect a DT_{50} > 16 days.

Under experimental conditions 1R-trans-Z-momfluorothrin underwent photodegradation with a half-life of 13.4 to 14.5 OECD solar days. However, information on photodegradation is difficult to

[1] Equation DT_{50} at x^{o}C = DT_{50} (t).e $^{(0.08.(T-x))}$ where t is experimental half life at T temperature

use for classification purposes given the actual degree of photodegradation in the aquatic environment depends on local conditions e.g. water depth, suspended solids, turbidity as well as seasonal influences.

In a ready biodegradation study minimal degradation (-5.46 to 3.91%) was observed.

In an aerobic water-sediment study 1R-trans-Z-momfluorothrin was removed rapidly with whole system DT_{50} values between 0.6 to 2.9 days. Various degradants were identified with longer DT_{50} values and mineralisation ranged from 15% AR at day 100 to 43% AR at day 105 in different systems. Data are not available on for the classification of such degradants.

Overall, the degradation information does not provide sufficient data to show 1R-trans-Z-momfluorothrin is ultimately degraded within 28 days (equivalent to a half-life < 16 days) or to non classifiable products. Consequently, 1R-trans-Z-momfluorothrin is considered not rapidly degradable for the purpose of classification and labelling.

4.2 Environmental distribution

4.2.1 Adsorption/Desorption

Two GLP studies are available investigation the adsorption of 1R-trans-Z-momfluorothrin (purity >99%).

The first study (Ponte, 2011b) followed OECD Guideline 121 with K_{oc} values of 7079 and 7413 determined. This equates to $\log K_{oc}$ values of 3.85 and 3.87.

The second study (Ponte, 2012b) followed OECD Guideline 106 using four soils and one sediment. The test was conducted using 1R-trans-Z-momfluorothrin and no significant further isomerisation was observed. There was a high correlation between adsorption and the soil organic carbon content and cation exchange capacity (CEC) indicating increased adsorption with increased organic carbon or CEC. There was no evidence of pH dependency. K_{oc} values ranged from 1033 to 4344 with an average of 1748. This equates to $\log K_{oc}$ values between 3 and 3.6 with an average of 3.2. The K_{oc} values for desorption ranged from 1085 to 6219 with an average of 2484.

Overall the two studies indicate 1R-trans-Z-momfluorothrin will be relatively immobile in soil/sediment.

4.2.2 Volatilisation

Experimental data (Moseley, 2001d; Leslie, 2011 and Moseley, 2011e) indicate the vapour pressure is between 4.702×10^{-7} and 4.702×10^{-7} Pa at 20°C for the RTZ and RTE isomers. The Henry's Law Constants (Foster, 2012c; Foster 2012d; Foster, 2012e) between 2.792×10^{-4} and 2.985×10^{-4} Pa m^3 mol^{-1} at 20°C indicating 1R-trans-Z-momfluorothrin is unlikely to partition from the water phase to air.

4.2.3 Distribution modelling

Not relevant for classification and labelling.

4.3 Aquatic Bioaccumulation

A summary of available information on the bioaccumulation potential of 1R-trans-Z-momfluorothrin is presented in Table 34 below.

Table 34: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
Partition coefficient *n*-octanol/water OECD Guideline 107, GLP	Log Pow: 2.99 at 25°C	Valid study RTZ isomer	Wright, 2011b
Partition coefficient *n*-octanol/water OECD Guideline 107, GLP	Log Pow: 2.88 at 25°C	Valid study RTE isomer	Wright, 2011c
Experimental aquatic BCF OECD Guideline 305, GLP	Steady state whole fish BCF: 600 to 612 l/kg Kinetic whole fish BCF: 753 to 784 l/kg (experimental 2.56% lipid). Lipid normalized to 5% kinetic whole fish BCF: 1471 to 1531 l/kg Depuration half-life DT_{50} whole fish: 9.76 to 11.7 days	Valid study Flow through, 35 days exposure, 28 days depuration Based on measured total radioactive [^{14}C] residues (TRR)	Kang, 2012

4.3.1 Aquatic bioaccumulation

4.3.1.1 Bioaccumulation estimation

As experimental data are available, estimations are not included.

4.3.1.2 Measured bioaccumulation data

An experimental aquatic BCF s available following GLP and OECD Guideline 305 (Kang, 2012). The study used [methoxymethylbenzyl-α-$_{14}$C] 1R-trans-E-momfluorothrin (purity >99%) and [methoxymethylbenzyl-α-$_{14}$C] 1R-trans-Z-momfluorothrin (purity > 99%) in a combined ratio of 1:9.

A flow-through system was used with Bluegill Sunfish (*Lepomis macrochirus*) and two exposure concentrations; 0.1 and 0.3 µg/l. The exposure period ran for 35 days followed by a 28-day depuration period. Test conditions reflected the guideline with a water pH 7.1-8.4. Samples were analysed by LSC and HPLC with radiometric detection. Water concentrations were considered stable.

1R-trans-Z/E-momfluorothrin was only detected in fish tissue on day 1 of the exposure phase in edible, non-edible and whole body fish samples at both low and high concentrations. After this it was considered metabolised and only [^{14}C] residues were observed. Major metabolites in fish were characterised as TFPA (detected from day 1 with maximum of 62 to 64.7% on day 3 in low and high doses) and MFOA-D (detected from day 14 with maximum of 2.6 to 4.7% on day 21 in low and high doses). Steady state and kinetic BCFs based on the parent 1R-trans-Z/E-momfluorothrin could not be calculated and BCFs were based on total radioactive residues (TRR).

Steady state (TRR) whole fish BCFs were 612 l/kg for the 0.1 µg/l nominal exposure concentration and 600 l/kg for the 0.3 µg/l nominal exposure concentration.

Kinetic whole fish BCFs were 753 l/kg for the 0.1 µg/l nominal exposure concentration and 784 l/kg for the 0.3 µg/l nominal exposure concentration. The higher concentration kinetic BCF was normalised to 5% lipid content wet weight considering an experimental lipid content of 2.56% giving BCFs of 1471 to 1531 l/kg at 5%.

At the end of the depuration period, TRR were less than 40%. Depuration half-lives (DT_{50}) for whole fish were calculated to be 9.76 to 11.7 days.

4.3.2 Summary and discussion of aquatic bioaccumulation

Experimental $logK_{ow}$ values are 2.88 and 2.99 at 25^{o}C for the RTE and RTZ isomers respectivley. These values are below the CLP $logK_{ow}$ trigger value of ≥ 4 intended to identify substances with a potential to bioaccumulate.

In an experimental BCF study, 1R-trans-Z-momfluorothrin was observed to be extensively metabolised resulting in a BCF less than 500. BCFs based on total radioactive residue were greater than 500 l/kg but less than 2000 l/kg reflecting metabolites. A clear depuration phase was observed.

4.4 Aquatic toxicity

A summary of available valid information on the aquatic toxicity of 1R-trans-Z-momfluorothrin is presented in Table 35 below. The studies used technical ‘S-1563’ with an overall purity of 95.4 to 95.8% (based on total momfluorothrin isomer content) and ≥ 86% 1R-trans-Z-momfluorothrin content.

No ecotoxicity data is available for degradants.

Table 35: Summary of relevant information on aquatic toxicity

Guideline / GLP status	Species	Endpoint	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity (mg/l)	
Acute toxicity to fish OECD Guideline 203, GLP	Rainbow Trout (*Oncorhynchus mykiss*)	Mortality	Flow-through	96 hours	LC_{50}	0.0012 (mm)	Fournier, 2011a
Acute toxicity to fish OECD Guideline 203, GLP	Fathead Minnow (*Pimephales promelas*)	Mortality	Flow-through	96 hours	LC_{50}	0.0097 (mm)	Fournier, 2011b
Acute toxicity to fish OECD Guideline 203, GLP	Bluegill Sunfish (*Lepomis macrochirus*)	Mortality	Flow-through	96 hours	LC_{50}	0.0029 (mm)	Fournier, 2011c
Fish Early Life-Stage (FELS) toxicity OECD Guideline 210, GLP	Fathead Minnow (*Pimephales promelas*)	Embryo hatching success; percentage embryos that produce live normal larvae at hatch; larval survival; and larval growth	Flow-through	28 days	NOEC	≥0.0031 (mm) Based on highest test concentration as no significant effects observed.	York, 2012
Daphnia sp Acute Immobilisation OECD Guideline, 202 GLP	*Daphnia magna*	Acute immobilisation	Flow-through	48 hours	EC_{50}	0.0078 (mm)	Fournier, 2011d
Daphnia magna Reproduction OECD Guideline 211, GLP	*Daphnia magna*	Survival; reproduction; growth	Flow-through	21 days	NOEC	0.0005 (mm)	Fournier, 2012
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP	*Pseudo-kirchneriella subcapitata*	Cell multiplication inhibition	Static	72 hours	ErC_{50} NOErC	>4.8 (twa) 0.33 (twa)	Softcheck, 2011a
Lemna sp. Growth Inhibition Test OECD Guideline 221, GLP	*Lemna gibba*	Growth	Static	7 days	ErC_{50} NOErC	>2.5 (twa) ≥2.5 (twa) Based on highest test concentration as no significant effects observed.	Softcheck, 2011b

mm refers to mean measured
twa refers to time weighted average

4.4.1 Fish

4.4.1.1 Short-term toxicity to fish

Three acute toxicity to fish studies using S-1563 are available following GLP and OECD Guideline 203.

Study 1 (Fournier, 2011b)

Using Rainbow Trout (*Oncorhynchus mykiss*), the nominal exposure range was 0.63, 1.3, 2.5, 5 and 10 µg/l. Exposure solutions were prepared with the aid of the solvent dimethylformamide (DMF) and a solvent control was included. Analytical measurement was determined using gas chromatography with electron capture detection (GC/ECD). Results were based on mean measured values: 0.64, 1.4, 2.2, 5 and 10 µg/l. Validity criteria were met and test is considered reliable. The 96 hour LC_{50} was calculated to be 1.2 µg/l (equating to 0.0012 mg/l) with 95% confidence intervals of 0.64 to 2.2 µg/l.

Study 2 (Fournier, 2011b)
Using Fathead Minnow (*Pimephales promelas*), the nominal exposure range was 1.3, 2.5, 5, 10 and 20 µg/l. Exposure solutions were prepared with the aid of the solvent DMF and a solvent control was included. Analytical measurement was determined using GC/ECD. Losses were observed at lower concentrations and results were based on mean measured values: 0.94, 2.1, 4, 9.5 and 18 µg/l. Validity criteria were met and test is considered reliable. The 96-hour LC_{50} was calculated to be 9.7 µg/l (equating to 0.0097 mg/l) with 95% confidence intervals of 7.7 to 13 µg/l.

Study 3 (Fournier, 2011c)
Using Bluegill Sunfish (*Lepomis macrochirus*), the nominal exposure range was 0.63, 1.3, 2.5, 5, and 10 µg/l. Exposure solutions were prepared with the aid of the solvent DMF and a solvent control was included. Analytical measurement was determined using GC/ECD. Results were based on mean measured values: 0.54, 1.0, 1.9, 4.1 and 8 µg/l. Validity criteria were met and test is considered reliable. The 96-hour LC_{50} was calculated to be 2.9 µg/l (equating to 0.0029 mg/l) with 95% confidence intervals of 2.5 to 3.4 µg/l.

Overall, the three acute toxicity to fish studies, using three different species, show LC_{50} values in the 0.001 to 0.01 mg/l range with the lowest 96-hour LC_{50} of 0.0012 mg/l.

4.4.1.2 Long-term toxicity to fish

A 28-day chronic toxicity to fish study (York, 2012) using S-1563 following GLP and OECD Guideline 210 is available. This fish early life stage study used Fathead Minnow (*Pimephales promelas*) which was not the most acutely sensitive species. The following endpoints: mean embryo hatching success, live normal larvae at hatch, mean larval survival, mean larval length and mean larval dry weight. The nominal exposure range was 0.1, 0.23, 0.64, 1.6, and 4 µg/l. Exposure solutions were prepared with the aid of the solvent DMF and a solvent control was included. Analytical measurement was determined using GC/ECD. Results were based on mean measured values: 0.1, 0.2, 0.48, 1.3, and 3.1 µg/l. Validity criteria were met and test is considered reliable. Endpoint test data are presented in Table 36. Overall, no significant difference could be determined

for any endpoint at any exposure concentration and the 28-day NOEC reflects the highest test concentration at 3.1 µg/l (equating to 0.0031mg/l) based on mean measured concentrations.

Table 36: Percent hatching success and percent normal larvae at completion of hatch (test day 4) and survival, total length and dry weight of larvae at test termination of the early life-stage (28 days post-hatch)

S-1563 **concentration**		**Mean embryo hatching success (%)**	**Live, normal larvae at hatch (%)**	**Mean larval survival (%)**	**Mean larval length (mm) (SD)[a]**	**Mean larval dry weight (mg) (SD)[a]**
Nominal (µg/l)	**Mean measured (µg/l)**					
Control	Control	81	100	98	27.2 (0.60)	38.6 (2.8)
Solvent control	Solvent control	80	100	93	27.2 (0.83)	38.5 (4.3)
Pooled control	Pooled control	80	100	96	27.2 (0.67)	38.5 (3.4)
0.10	0.10	77	99	97	26.5 (0.46)	36.0 (1.9)
0.26	0.20	74	99	97	26.7 (0.46)	36.3 (1.8)
0.64	0.48	79	100	92	27.3 (0.78)	38.9 (4.3)
1.6	1.3	76	100	95	26.9 (0.38)	37.2 (1.4)
4.0	3.1	78	97[b]	92	26.8 (0.46)	36.9 (0.7)

[a] (SD) = Standard Deviation

[b] Significantly reduced compared to pooled control, based on Wilcoxon's Test with Bonferroni's Adjustment. However, the 3% difference between the pooled control and the high exposure concentration falls within the normal variability for fathead minnows; therefore, the statistical difference between the pooled control and the high exposure concentration was determined to not be biologically relevant.

4.4.2 Aquatic invertebrates

4.4.2.1 Short-term toxicity to aquatic invertebrates

An acute toxicity to *Daphnia magna* study (Fournier, 2011d) using S-1563 is available following GLP and OECD Guideline 202. Exposure solutions were prepared with the aid of the solvent DMF and a solvent control was included. The nominal exposure range was 4, 8, 16, 32, and 64 µg/l. Analytical measurement was determined using GC/ECD. Results were based on mean measured values: 3.6, 7.9, 17, 28 and 61µg/l. Validity criteria were met and test is considered reliable. The 48-hour EC_{50} was calculated to be 7.8 µg/l (equating to 0.0078 mg/l) with 95% confidence intervals of 6.1 to 9.7 µg/l.

4.4.2.2 Long-term toxicity to aquatic invertebrates

A chronic toxicity to *Daphnia magna* study (Fournier, 2012) using S-1563 is available following GLP and OECD Guideline 211. Exposure solutions were prepared with the aid of the solvent DMF and a solvent control was included. The nominal exposure range was 0.082, 0.20, 0.51, 1.3, 3.2, and 8 µg/l. Analytical measurement was determined using GC/ECD. Results were based on mean measured values: 0.074, 0.21, 0.5, 1.3, 3.1 and 9.3 µg/l. Validity criteria were met and test is considered reliable. Endpoint test data is presented in Table 37 with endpoint NOEC values presented in Table 38.

Table 37: Survival, reproduction and growth data at day 21

S-1563 **concentration**		**Mean cumulative percent survival of parents (%) (SD)[a]**	**Mean cumulative number of offspring per female (%) (SD)[a]**	**Mean total body length of parents (mm) (SD)[a]**	**Mean dry weight of parents (mg) (SD)[a]**
Nominal (µg/l)	**Measured (µg/l)**				
Control	Control	85 (6)	106 (16)	4.14 (0.15)	0.71 (0.12)
Solvent control	Solvent control	95 (6)	156 (6)	4.55 (0.03)	0.96 (0.07)
Pooled control	Pooled control	90 (8)	n.a.[b]	n.a.	n.a.
0.082	0.074	95 (6)	182 (6)	4.64 (0.03)	1.04 (0.04)
0.20	0.21	90 (20)	155 (11)	4.46 (0.08)	0.84 (0.16)
0.51	0.50	100 (0)	141 (8)	4.44[de] (0.05)	0.86 (0.06)
1.3	1.3	90 (20)	127[ce] (24)	4.20[de] (0.20)	0.63[c] (0.17)
3.2	3.1	90 (0)	155 (11)	4.51 (0.14)	0.68[c] (0.15)
8.0	9.3	73[f] (42)	25[c] (4)	3.48[d] (0.29)	0.39[c] (0.06)

[a] (SD) = Standard Deviation
[b] n.a. = not applicable. Treatment data were compared to the solvent control to determine effects for this endpoint
[c] Significantly reduced compared to the solvent control, based on Dunnett's Multiple Comparison.
[d] Significantly reduced compared to the solvent control, based on Steel's Many-One Rank Test. Due to the lack of statistical significance at treatment level (3.1 µg/l), the effects observed at the 0.50 and 1.3 µg/l treatment levels were determined to not be toxicant-related.
[e] Due to the lack of statistical significance at treatment level (3.1 µg/l), the effect observed at the 1.3 µg/l treatment level was determined to not be toxicant-related.
[f] Survival data did not meet the assumption fit a normal distribution normality, but did meet the assumption for homogeneity of variance. Therefore, survival data were evaluated using Wilcoxon's Test with Bonferroni's Adjustment (U.S. EPA, 2002), a non-parametric procedure, to establish treatment effects. This statistical analysis determined no significant difference in percent survival among daphnids exposed to any of the treatment levels tested compared to the pooled control data (i.e., 90%).

Table 38: NOEC data based on mean measured concentrations

Endpoint	**NOEC (µg/l)**
21–day survival	9.3
21-day reproduction	3.1
21-day total body length	3.1
21-day dry weight	0.50

The lowest study 21-day NOEC was 0.5 µg/l (equating to 0.0005 mg/l) based on total body dry weight. While statistical differences were observed between the 0.5 and 1.3 µg/l treatments and controls for parental body length, these were considered not due to the test substance due to a lack of clear dose-response. The UK CA notes that the resulting NOEC (0.21 µg/l), should such effects be taken into account, would be in the same 0.0001 to 0.001 mg/l range as the quoted study NOEC for the purpose of classification.

4.4.3 Algae and aquatic plants

Algae:

An algal growth inhibition study (Softcheck, 2011a) using S-1563 and *Pseudokirchneriella subcapitata* is available following GLP and OECD Guideline 201. Exposure solutions were prepared with the aid of the solvent DMF (dimethylformamide) and a solvent control was included.

The nominal exposure range was 0.19, 0.38, 0.75, 1.5, 3 and 6 mg/l. Analytical measurement was determined using GC/ECD. Results were based on time-weighted average measured values: 0.17, 0.33, 0.64, 1.3, 2.6 and 4.8 mg/l. Validity criteria were met and test is considered reliable.

At 72 hours 21% growth inhibition was observed at the highest exposure concentration meaning the E_rC_{50} was considered >4.8 mg/l. The 72-hour NOE_rC was determined to be 0.33 mg/l.

Aquatic plants:

A 7-day toxicity to *Lemna gibba* study (Softcheck, 2011b) using S-1563 is available following GLP and OECD Guideline 221. Exposure solutions were prepared with the aid of the solvent DMF and a solvent control was included. The nominal exposure range was 0.38, 0.75, 1.5, 3 and 6 mg/l. Analytical measurement was determined using GC/ECD. Results were based on time-weighted average measured values: 0.26, 0.53, 1, 1.9, and 2.5 mg/l. Validity criteria were met and test is considered reliable. The study endpoints were percentage reduction in frond density, dry weight biomass, growth rate based on frond density, yield and growth rate based on dry weight.

At 7 days, the percentage inhibition of growth rate based on frond density and dry weight was 4 and 5 % respectively at the highest exposure concentration of 2.5 mg/l. Therefore, the E_rC_{50} was considered >2.5 mg/l. As no significant reduction compared to controls was observed, the 7-day growth NOE_rC is considered to be $\geq$ 2.5 mg/l.

4.4.4 Other aquatic organisms (including sediment)

No aquatic exposure data available.

4.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

For the purpose of classification, 1R-trans-Z-momfluorothrin is considered not rapidly degradable.

1R-trans-Z-momfluorothrin has log Kow values below 4. In a fish bioaccumulation study 1R-trans-Z-momfluorothrin was extensively metabolised resulting in a BCF below 500.

Aquatic acute toxicity data are available for fish, invertebrates, algae and aquatic plants. Fish and invertebrates are the most sensitive trophic group with $L(E)C_{50}$ values in the range 0.001 to 0.01 mg/l. The lowest value is a 96-h LC_{50} of 0.0012 mg/l for fish. 1R-trans-Z-momfluorothrin should be classified as Aquatic Acute 1 with an M factor of 100.

Chronic toxicity data are available for fish, invertebrates, algae and aquatic plants. The lowest value is a 21-day NOEC for *Daphnia magna* of 0.0005 mg/l. Given this is the range 0.0001 to 0.001 mg/l, 1R-trans-Z-momfluorothrin should be classified as Aquatic Chronic 1 with an M factor of 100. The UK CA notes that while acute LC_{50} values are available for three fish species, the species used for the single chronic fish test did not reflect the most sensitive fish species resulting in a chronic NOEC greater than the LC_{50} values for two fish species. On this basis, it is appropriate to consider the surrogate approach for chronic toxicity to fish. This also results in a classification of Aquatic Chronic 1 with an M factor of 100. The UK CA notes that 1R-trans-Z-momfluorothrin is an insecticide and data are not available for this class of invertebrates. Therefore, it is possible that more stringent M factors may be applicable if such data were to be available in the future.

4.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

Aquatic Acute 1; H400: Very toxic to aquatic life

Acute M factor = 100

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

Chronic M factor = 100

RAC evaluation of environmental hazards

Summary of the Dossier submitter's proposal

1R-trans-Z-momfluorothrin does not currently have a harmonised classification for environmental hazards. Based on the available data on aquatic toxicity and considering that the substance is not rapidly degradable, the dossier submitter (DS) proposed an environmental classification as Aquatic Acute 1 (M=100) and Aquatic Chronic 1 (M=100) according to the CLP Regulation.

Degradation

Hydrolysis

1R-trans-Z-momfluorothrin is considered stable under acidic conditions with limited hydrolysis at neutral pH. Under alkaline conditions, degradation is significant although it is likely to reflect a DT_{50} > 16 days.

The available hydrolysis study (following GLP and according to OECD TG 111) used 3 radio-labelled (^{14}C) 1R-trans-Z-momfluorothrin isomers. In a preliminary test, hydrolysis was assessed at pH 4, 7 and 9 at 50 °C for up to 5 days. The acid isomers used were stable at pH 4 and limited degradation occurred at pH 7 and 9. The definitive study was performed at pH 7 (up to 33 days for 40, 50 and 60°C) and pH 9 (up to 21 days for 25, 40 and 50°C). The calculated DT_{50} were: 660 to 1394 days at 20°C (pH 7) and 11.7 to 12.2 days at 20°C (pH 9). However, the DT_{50} converted to a more environmentally relevant temperature of 12°C were 18.3 to 20.3 days at pH 9.

Photolysis

An aqueous photolysis study was carried out, using a Xenon lamp, and 3 radio-labelled (^{14}C) 1R-trans-Z-momfluorothrin isomers, at pH 4 and 25 °C for 13 days, following GLP and OECD TG 316. Under light conditions, the isomerisation was not significantly different between the 3 labelled (^{14}C) 1R-trans-Z-momfluorothrin isomers. The photolytic DT_{50} of 1R-trans-Z-momfluorothrin was determined to be 13.4 days, equivalent to 25.9 OECD solar days and, including photoisomerisation, 7.5 days equivalent to 14.5 OECD solar days.

Biodegradation

Ready biodegradability was tested with one study following GLP and OECD TG 301B (CO_2 Evolution Test), using S-1563 (purity 95.6%, total momfluorothrin isomer content) as test material. The study was run a pH 7.53 to 7.68 and between 21 and 24°C. Validation criteria for the reference and toxicity controls were met. Ultimate biodegradation reached a maximum of 3.91%, demonstrating that the substance is not readily biodegradable.

A degradation study in a water-sediment system is available which followed OECD TG 308 (Aerobic and Anaerobic Transformation in Aquatic Sediment Systems) and was GLP-compliant. Two aerobic systems were used. The study used 3 radio-labelled (^{14}C) 1R-

trans-Z-momfluorothrin isomers. Initial nominal dose rates were 7.7 to 8.4 µg per sample with direct addition to the water phase. The study was run in the dark at 20 ± 2°C for between 100 and 146 days depending on the radio-label.

1R-trans-Z-momfluorothrin was removed rapidly, with whole system DT_{50} values between 0.6 to 2.9 days. Various degradants were identified with longer DT_{50} values and mineralisation ranged from 15% AR at day 100 to 43% AR at day 105 in different systems. Data are not available on the classification of such degradants.

Degradation information does not provide sufficient data to show that 1R-trans-Z-momfluorothrin is ultimately degraded within 28 days (equivalent to a half-life < 16 days) or to non-classifiable products. Consequently, 1R-trans-Z-momfluorothrin is considered not rapidly degradable for the purpose of classification and labelling.

Aquatic bioaccumulation

A summary of available information on the bioaccumulation potential of 1R-trans-Z-momfluorothrin is presented in the Table below.

Method	Results	Remark	Reference
Partition coefficient n-octanol/water OECD TG 107, GLP	Log Kow: 2.99 at 25°C	RTZ isomer	Wright, 2011b
Partition coefficient n-octanol/water OECD TG 107, GLP	Log Kow: 2.88 at 25°C	RTE isomer	Wright, 2011c
Experimental aquatic BCF OECD TG 305, GLP	Steady state whole fish BCF: 600 to 612 L/kg Kinetic whole fish BCF: 753 to 784 L/kg (experimental 2.56% lipid). Lipid normalized to 5% kinetic whole fish BCF: 1471 to 1531 L/kg Depuration half-life DT_{50} whole fish: 9.76 to 11.7 days	Flow through, 35 days exposure, 28 days depuration Based on measured total radioactive [14C] residues (TRR)	Kang, 2012

Experimental $logK_{OW}$ values are 2.88 and 2.99 at 25°C for the RTE and RTZ isomers, respectivley.

These values are below the $logK_{OW}$ trigger value of 4 intended to identify substances with a potential to bioaccumulate according to CLP.

An experimental aquatic BCF is available following GLP and OECD TG 305. The study used 2 radio labels (^{14}C) momfluorothrin isomers in a combined ratio of 1:9. A flow-through system was used with Bluegill Sunfish (*Lepomis macrochirus*) and two exposure concentrations: 0.1 and 0.3 µg/L. The exposure period ran for 35 days followed by a 28 day depuration period. Test conditions reflected the OECD TG with a water pH between 7.1-8.4.

1R-trans-Z/E-momfluorothrin was only detected in fish tissue on day 1 of the exposure phase in edible, non-edible and whole body fish samples at both low and high concentrations. After this, it was considered metabolised and only [^{14}C] residues were observed. Steady state and kinetic BCFs based on the parent 1R-trans-Z/E-momfluorothrin could not be calculated and the provided BCFs were based on total radioactive residues, related to the major metabolites detected in fish (TFPA and MFOA-D).

In conclusion, momfluorothrin was observed to be extensively metabolised, resulting in a BCF less than 500. The provided BCFs, based on total radioactive residue, were greater than 500 L/kg but less than 2000 L/kg, reflecting metabolites. A clear depuration phase was observed.

Aquatic toxicity

Several acute and chronic aquatic toxicity studies conducted following GLP and standard test guidelines are available. The studies used technical 'S-1563' with an overall purity of 95.4 to 95.8% (based on total momfluorothrin isomer content) and ≥ 86% 1R-trans-Z-momfluorothrin isomer content.

No ecotoxicity data are described in the CLH report for the degradants. However the DS made reference to non-GLP acute toxicity studies with algal, *Daphnia* and fish (Miyamoto *et al*., 2013a, 2013b and 2013c) for the degradants MFOA-D, MFOA and t-COOH-CA, indicating they are significantly less toxic than the parent.

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

Method	**Test organism**	**Test system**	**Endpoint mg/l**	**Remarks**	**Reference**
OECD 203, GLP	*Oncorhynchus mykiss*	96 h Flowthrough	**LC_{50} 0.0012**	mm	Fournier, 2011a
OECD 203, GLP	*Pimephales promelas*	96 h Flowthrough	LC_{50} 0.0097	mm	Fournier, 2011b
OECD 203, GLP	*Lepomis macrochirus*	96 h Flowthrough	LC_{50} 0.0029	mm	Fournier, 2011c
OECD 210, GLP	*Pimephales promelas*	28 d Flowthrough	NOEC ≥ 0.0031	mm Based on highest test concentration as no significant effects observed.	York, 2012
OECD 202, GLP	*Daphnia magna*	48 h Flowthrough	EC_{50} 0.0078	mm	Fournier, 2011d
OECD 211, GLP	*Daphnia magna*	21 d Flowthrough	**NOEC 0.0005**	mm	Fournier, 2012
OECD 201, GLP	*Pseudokirchneriella subcapitata*	72 h Static	ErC_{50} >4.87 NOErC 0.33	twa	Softcheck, 2011a
OECD 221, GLP	*Lemna gibba*	7 d Static	ErC_{50} >2.5 NOErC ≥2.5	twa Based on the highest test concentration as no significant effects were observed.	Softcheck, 2011b
mm = mean measured twa = time weighted average					

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

mg/L is based on mean measured concentrations, with 95% confidence intervals of 0.64 to 2.2 µg/L.

Based on chronic aquatic toxicity data, the lowest NOEC was for invertebrates in the range of 0.0001 to 0.001 mg/L. The most sensitive species tested is *Daphnia magna,* (21d flowthrough condition test) with a NOEC of 0.0005 mg/L, based on total body dry weight.

Comments received during public consultation

Three MSCAs and one industry representative contributed during public consultation stating a general agreement with the proposed environmental classification.

One MSCA suggested to recalculate the hydrolysis half-lives by application of the recommended EU outdoor temperature of 285 K (12°C); and to indicate the metabolites identified during the hydrolysis study and the aqueous photolysis of the parent as well as their quantified maximum percentages. The DS replied that since hydrolysis is pH dependant (increasing hydrolysis with increasing pH), the values presented in the CLH report (DT_{50} of 18.3 to 20.3 days at pH 9 and 12°C) were considered to represent the most rapid hydrolysis at a higher environmentally relevant pH range. The DS provided the % AR of the principal degrandants. However, other details about the degradants were not presented in the CLH report as the parent is considered to be more toxic than the degradation products and so the classification proposal focused on the parent substance alone.

The same MSCA provided some minor comments referring to the water/sediment study (mineralisation data, the temperature of all DT_{50} values, the maximum % recovery rates for major degradants). The DS replied that all relevant information was provided in the CLH report.

They aslo asked to provide a chapter on fate and behavior in atmosphere including results on indirect phototransformation in air. The DS replied that 1R-trans-Z-momfluorothrin is unlikely to partition to the atmosphere and environmental classification does not include consideration of the air compartment, with exception of substances hazardous to ozone layer.

A second MS pointed out to an editorial comment on the vapour pressure value and the DS agreed.The industrial representative proposed some editorial comments on the water-sediment simulation study, to which the DS agreed. They also suggested to consider an additional aquatic acute toxicity study of 2 degradants to fish, Daphnia and algae. The DS replied that since the studies indicate the degradants to be significantly less toxic than the parent and because 1R-trans-Z-momfluorothrin is considered not rapidly degradable, they were not used further and the CLH report focuses on the parent alone.

The industrial representative also proposed to consider appropriate the NOEC value of 0.50 µg/L. They argued that since the mean total body length at 0.50 µg/L (i.e. 4.44 mm) is within the variation range of control (4.14 mm) and solvent control (4.55 mm), the statistically significant difference observed at 0.50 µg/L for the length is not considered related to a toxicity effect. The DS replied the NOEC value for the parental body length parameter is 0.0031 mg/L. Conequently, should the statistical difference be valid for NOEC derivation, a resulting parental body length NOEC would, in any case, fall within the same range of the CLH criteria (0.0001 to 0.001 mg/L) as the dry weight NOEC and would have no impact on the classification proposal. On this basis the endpoint was not considered further for classification.

The industrial representative suggested to consider an additional toxicity test with sediment-dwellig midges. The DS clarified that the proposed test it isn't an aquatic exposure study and therefore not appropriate, to be included in the CLH report.

Assessment and comparison with the classification criteria

Degradation

According to all the provided information on degradation, RAC agrees with the DS proposal to consider 1R-trans-Z-momfluorothrin not readily biodegradable and not rapidly degradable.

Bioaccumulation

1R-trans-Z-momfluorothrin has log Kow values below 4. In a fish bioaccumulation study the provided BCFs were related to the metabolites, because the parental 1R-trans-Z-momfluorothrin was only detected in fish tissue on day 1 of the exposure phase and afther this it was considered extensively metabolised. Based on these results, a BCF less than 500 could be applied.

Aquatic toxicity

Acute aquatic hazard

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

RAC concludes that 1R-trans-Z-momfluorothrin should therefore be classified as Aquatic Acute 1 (H400), with an M-factor of 100.

Chronic aquatic hazard

The long-term aquatic toxicity data are available for all three trophic levels. The lowest value is a 21 d NOEC=0.0005 mg/L (mean measured concentration) for *Daphnia magna.* This NOEC value is in the range of 0.0001 to 0.001 mg/L. Therefore, RAC concludes that 1R-trans-Z-momfluorothrin should be classified as Aquatic Chronic 1 (H410), with an M-factor of 100.

RAC notes that the species used for the single chronic fish test did not reflect the most sensitive fish species (*Oncorhynchus mykiss)* for the acute values, resulting in a chronic NOEC greater than the LC_{50} values for two fish species. On this basis, it is appropriate to consider the surrogate approach for chronic toxicity to fish. This also results in a classification of Aquatic Chronic 1 (H410), with an M-factor of 100, based on the lowest chronic aquatic toxicity value and the fact that 1R-trans-Z-momfluorothrin is not rapidly degradable.

In summary, RAC agrees with the DS proposal that 1R-trans-Z-momfluorothrin should be classified according to CLP as:

Aquatic Acute 1 (H400), M-factor of 100;
Aquatic Chronic 1 (H410), M-factor of 100.

5 OTHER INFORMATION

None.

6 REFERENCES

Physico-chemical properties

Author(s)	Year	Title.
Moseley, R. H.	2011a	S-1563PAI: Determination of melting/boiling point. Covance Laboratories Ltd., Otley Road, Harrogate, North Yorkshire, HG3 1PY, UK. Report No. 8243288 [Sumitomo Ref: RWP-0015] GLP Unpublished
Moseley, R. H.	2011b	S-1563RTZ: Determination of melting/boiling point. Covance Laboratories Ltd., Otley Road, Harrogate, North Yorkshire, HG3 1PY, UK. Report No. 8226992 [Sumitomo Ref: RWP-0017] GLP Unpublished
Foster, B.	2012a	S-1563PAI: Determination of relative density. Covance Laboratories Ltd., Otley Road, Harrogate, North Yorkshire, HG3 1PY, UK. Report No. 8226994 [Sumitomo Ref: RWP-0035] GLP Unpublished
Moseley, R. H.	2011d	S-1563: Evaluation of vapour pressure. Covance Laboratories Ltd., Otley Road, Harrogate, North Yorkshire, HG3 1PY, UK. Report No. 8226998 [Sumitomo Ref: RWP-0010] GLP Unpublished
Leslie, S.	2011	S-1563RTZ: Evaluation of vapour pressure. Covance Laboratories Ltd., Otley Road, Harrogate, North Yorkshire, HG3 1PY, UK. Report No. 8239095 [Sumitomo Ref: RWP-0011] GLP Unpublished
Foster, B.	2012c	S-1563PAI: Calculation of Henry's Law constant. Smithers Viscient (ESG) Ltd., Otley Road, Harrogate, North Yorkshire, HG3 1PY, UK. Report No. 8263536 [Sumitomo Ref: RWP-0041] GLP Unpublished
Foster, B.	2012e	S-1563RTZ: Calculation of Henry's Law constant. Smithers Viscient (ESG) Ltd., Otley Road, Harrogate, North Yorkshire, HG3 1PY, UK. Report No. 8263826 [Sumitomo Ref: RWP-0043] GLP Unpublished
Foster, B.	2012d	S-1563RTE: Calculation of Henry's Law constant. Smithers Viscient (ESG) Ltd., Otley Road, Harrogate, North Yorkshire, HG3 1PY, UK. Report No. 8263825 [Sumitomo Ref: RWP-0042] GLP Unpublished
Foster, B.	2012g	S-1563TGAI: Determination of Physical State, colour and odour. Smithers Viscient (ESG) Ltd., Otley Road, Harrogate, North Yorkshire, HG3 1PY, UK. Report No. 8226981 [Sumitomo Ref: RWP-0040] Not GLP

Author(s)	Year	Title.
		Unpublished
Leslie, S.	2010a	S-1563: Development and validation of an analytical method, and evaluation of the water solubility. Covance Laboratories Ltd., Otley Road, Harrogate, North Yorkshire, HG3 1PY, UK. Report No. 8226997 [Sumitomo Ref: RWP-0006] GLP Unpublished
Leslie, S.	2010b	S-1563RTZ: Development and validation of an analytical method, and evaluation of the water solubility. Covance Laboratories Ltd., Otley Road, Harrogate, North Yorkshire, HG3 1PY, UK. Report No. 8228970 [Sumitomo Ref: RWP-0007] GLP Unpublished
Wright, D.	2011a	S-1563RTZ: Evaluation of dissociation constant. Covance Laboratories Ltd., Otley Road, Harrogate, North Yorkshire, HG3 1PY, UK. Report No. 8226995 [Sumitomo Ref: RWP-0022] GLP Unpublished
Wright, D.	2011b	S-1563RTZ: Determination of octanol:water partition coefficient. Covance Laboratories Ltd., Otley Road, Harrogate, North Yorkshire, HG3 1PY, UK. Report No. 8226996 [Sumitomo Ref: RWP-0024] GLP Unpublished
Wright, D.	2011c	S-1563RTE: Determination of octanol:water partition coefficient. Covance Laboratories Ltd., Otley Road, Harrogate, North Yorkshire, HG3 1PY, UK. Report No. 8239094 [Sumitomo Ref: RWP-0025] GLP Unpublished
Foster, B.	2012j	S-1563TGAI: Determination of flammability and auto-flammability. Covance Laboratories Ltd., Otley Road, Harrogate, North Yorkshire, HG3 1PY, UK. Report No. 8226984 [Sumitomo Ref: RWP-0039] GLP Unpublished
Foster, B.	2011b	S-1563TGAI: Determination of surface tension. Covance Laboratories Ltd., Otley Road, Harrogate, North Yorkshire, HG3 1PY, UK. Report No. 8250878 [Sumitomo Ref: RWP-0021] GLP Unpublished
Foster, B.	2012k	S-1563TGAI: Assessment of explosive properties. Smithers Viscient (ESG) Ltd., Otley Road, Harrogate, North Yorkshire, HG3 1PY, UK. Report No. 8226985 [Sumitomo Ref: RWP-0044] GLP Unpublished
Foster, B.	2011c	S-1563TGAI: Assessment of oxidising properties. Covance Laboratories Ltd., Otley Road, Harrogate, North Yorkshire, HG3 1PY, UK. Report No. 8226983 [Sumitomo Ref: RWP-0020] GLP Unpublished

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1R-TRANS-Z-MOMFLUOROTHRIN

Human Health

Author(s)	Year	Title.
Deguchi, Y.	2010a	Acute oral toxicity study of S-1563 in rats. Report No. 4166 [Sumitomo Ref: RWT-0010] GLP Unpublished
Deguchi, Y.	2010b	Acute dermal toxicity study of S-1563 in rats. Report No. 4167 [Sumitomo Ref: RWT-0017] GLP Unpublished
Deguchi, Y.	2010c	Acute inhalation toxicity study of S-1563 in rats. Report No. 4168 [Sumitomo Ref: RWT-0021] GLP Unpublished
Ota, M.	2011a	Eye irritation test of S-1563 in rabbits. Report No. 4140 [Sumitomo Ref: RWT-0014] GLP Unpublished
Ota, M.	2011b	Skin irritation test of S-1563 in rabbits. Report No. 4141 [Sumitomo Ref: RWT-0015] GLP Unpublished
Ota, M.	2010	Skin sensitization test of S-1563 in guinea pigs (Maximization test). Report No. 4196 [Sumitomo Ref: RWT-0012] GLP Unpublished
Hadfield, N.	2012	S-1563 – *In Vitro* absorption from a 1% S-1563 formulation through human epidermis using [^{14}C]-S-1563. Report No. JV2161-REG [Sumitomo Ref: RWM-0013] GLP Unpublished
Mikata, K., Tarui, H.	2010	Amended Final Report 1: The Disposition and metabolism of S-1563RTZ and S-1563RTE in rats (a single administration) - English translation 2012. Sumitomo Chemical Co. Ltd Report No. S1473 [Sumitomo Ref: RWM-0009] GLP Unpublished
Hayashi, K. Tarui, H.	2009	Metabolism of S-1563RTZ in rats - distribution, metabolism and excretion in rats after repeated oral administration – English translation 2012. Report No. P090160 [Sumitomo Ref: RWM-0001] GLP Unpublished
Tarui, H.	2012	*In vitro* metabolism of S-1563RTE and S-1563RTZ in rat and human liver microsome. Sumitomo Chemical Co. Ltd. Report No. X0177 [Sumitomo Ref: RWM-0017] Not GLP Unpublished

Braun, L.	2008	S-1563: 2-week oral (capsule) toxicity study in the beagle dog. Report No. B87985, [Sumitomo Ref. RWT-0001] Not GLP Unpublished
Ogata, H.	2012	A 28-day repeat dose dermal toxicity study of S-1563 in rats. Study No. P111028 [Sumitomo Ref: RWT-0065] GLP Unpublished
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7 ANNEXES

Annex I: An Evaluation of the Human Relevance of S-1563-induced Liver Tumours in Rats Based on Mode of Action