CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Epsilon-metofluthrin

EC Number: **Not assigned**

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

Index Number: **Not assigned**

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	Metofluthrin
EC number:	***None assigned***
CAS number:	240494-71-7 [240494-70-6 – metofluthrin (all isomers)]
Annex VI Index number:	None assigned
Degree of purity:	75-89.4% [93-98.8 % (all metofluthrin isomers)]
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

	CLP Regulation
Current entry in Annex VI, CLP Regulation	Not currently listed
Current proposal for consideration by RAC	Acute Tox 3; H301 - Toxic if swallowed Acute Tox 4; H332 - Harmful if inhaled STOT-RE 2; H373 - May cause damage to organs through prolonged or repeated exposure (inhalation))

	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 Regulation)	Acute Tox 3; H301 - Toxic if swallowed Acute Tox 4; H332 - Harmful if inhaled STOT-RE 2; H373 - May cause damage to organs through prolonged or repeated exposure (inhalation)) 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 according to the CLP Regulation

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	**Category 3; H301 – Toxic if swallowed**	Not applicable	Not classified	Not Applicable
	Acute toxicity - dermal	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
	Acute toxicity - inhalation	**Category 4; H332 – Harmful if inhaled**	Not applicable	Not classified	Not Applicable
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	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	**STOT-RE 2: H373 – May cause damage to organs through prolonged or repeated exposure (inhalation)**	Not applicable	Not classified	Not Applicable

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 life** **Aquatic Chronic 1; H410 – Very toxic to aquatic life with long lasting effects**	Acute M=100 Chronic M=100	Not classified	Not applicable
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.

H332; Harmful if inhaled

H373: May cause damage to organs through prolonged or repeated exposure (inhalation)

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

Epsilon-metofluthrin[1] is a biocidal active substance that has been approved under Dir 98/8/EC (Implementing Directive 2010/71/EU of 4th November 2010).

In accordance with Article 36(2) of CLP, Epsilon-metofluthrin should now be considered for harmonised classification and labeling. Epsilon-metofluthrin does not currently have a harmonised classification in Annex VI of the CLP Regulation and the classification has not been considered previously.

This report provides a classification and labeling proposal based mainly on the information presented in the assessment of Epsilon-metofluthrin under Directive 98/8/EC.

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

2.2 Short summary of the scientific justification for the CLH proposal

Epsilon-metofluthrin is a biocidal active substance that has been approved under Dir 98/8/EC.

In a standard acute oral toxicity study in the rat, the LD_{50} was determined to be >2000 mg/kg following administration of the neat substance. However, in an acute neurotoxicity study, mortalities were observed in 7/20 rats at the top does of 100 mg/kg. In this latter study, the substance was administered in a corn oil vehicle. In a standard acute inhalation toxicity study in the rat, the 4-hour LC50 was found to be between 1-2 mg/l. Comparing the available data to the classification criteria, classification with Acute Tox 3; H301 – Toxic if swallowed and Acute Tox 4 – Harmful if inhaled is proposed,

Following repeated inhalation exposure of rats to the substance at a concentration of 0.2 mg/l ,3h/d, 7d/week for 28 days, mortalities occurred throughout the treatment period (7/10 males and 3/10 females). In addition, tremor, suggestive of neurotoxicity was also observed. As such, classification with STOT-RE 2; H373 – May cause damage to organs through prolonged or repeated exposure (inhalation) is proposed.

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 Epsilon-metofluthrin causes tumours in rats by a

[1] NB; during the original review of the active substance under Directive 98/8/EC the substance was referred to by the ISO name Metofluthrin and not Epsilon-metofluthrin. Please refer to section 1.2 for full information on the substance identity and history.

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 groups. The most sensitive endpoint is in the fish, with 96-h LC_{50} of 0.0012 mg/l. The substance should be classified as Aquatic Acute 1; H400 – very toxic to aquatic life, with an acute M factor of 100.

The only chronic data point is the 72-h NOEC from the algal test. As this taxanomic group is clearly less sensitive that fish or invertebrates in acute tests, this means there are insufficient chronic data for classification and the surrogate approach should be used. As the L(E)C50 values are < 1 mg/l and the substance is not rapidly degradeable, classification with Aquatic Chronic 1; H410 – Very toxic to aquatic life with long lasting effects is appropriate, with a chronic M factor of 100.

2.3 Current harmonised classification and labelling

Not applicable, not currently listed on Annex VI of CLP.

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

Acute Tox 4: H332 – Harmful if inhaled

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

Epsilon-metofluthrin is a new biocidal active substance and, in accordance with Article 36(2) of CLP, should now be considered for harmonised classification and labelling.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

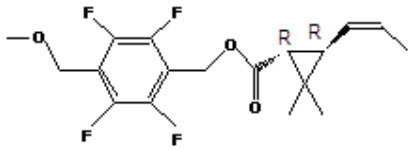
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	None assigned
EC name:	None assigned
CAS number (EC inventory):	Not listed
CAS number:	240494-71-7 (240494-70-6 - All 'metofluthrin' isomers)
CAS name:	Cyclopropanecarboxylic acid, 2,2-dimethyl-3-(1Z)-1-propen-1-yl-, [2,3,5,6-tetrafluoro-4-(methoxymethyl)phenyl]methyl ester, (1R,3R)- [ALL ISOMERS - Cyclopropanecarboxylic acid, 2,2-dimethyl-3-(1-propen-1-yl)-, [2,3,5,6-tetrafluoro-4-(methoxymethyl)phenyl]methyl ester]
IUPAC name:	2,3,5,6-tetrafluoro-4-(methoxymethyl)benzyl (1R,3R)-2,2-dimethyl-3-[(1Z)-prop-1-en-1-yl]cyclopropanecarboxylate [ALL ISOMERS 2,3,5,6-tetrafluoro-4-(methoxymethyl)benzyl 2,2-dimethyl-3-(prop-1-en-1-yl)cyclopropanecarboxylate]
CLP Annex VI Index number:	Not listed
Molecular formula:	$C_{18}H_{20}F_4O_3$
Molecular weight range:	360.35

Structural formula:



1.2 Composition of the substance

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Metofluthrin		75.4-89.4% 93-98.8% (all 'metofluthrin' isomers)	

Current Annex VI entry: None

The ISO name Metofluorothrin is associated with the IUPAC name 2,3,5,6-tetrafluoro-4-methoxymethylbenzyl (EZ)-(1RS,3RS:1SR:3SR)-2,2-dimethyl-3-(prop-1-enyl) cyclopropancarboxylate. 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 2,3,5,6-tetrafluoro-4-methoxymethylbenzyl (Z)-(1R,3R) -2,2-dimethyl-3-(prop-1-enyl) cyclopropancarboxylate (CAS 240494-71-7), which is individually present at ≥ 75.4%. This has been referred to as the 'RTZ' isomer by the applicant and is now identified by the ISO name Epsilon-metofluthrin. The other 7 isomers are all individually present at concentrations > 0.1% but < 12%, with the total isomer content (i.e., metofluthrin content) ≥ 93%.

Under the original biocides assessment, the ISO name Metofluthrin was used to identify this substance. However, the purity of the RTZ isomer was given as ≥ 75.4%, with an overall metofluthrin isomer content of ≥ 93%. This was specified in the Implementing Directive 2010/71/EU of 4th November 2010. For clarity and ease of reference with the existing biocides CAR, the substance is referred to as metofluthrin in the physical, human health and environmental hazard sections of the CLH report.

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

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Confidential refer to IUCLID			

There are a number of 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 Dir 98/8/EC, the tested substance was considered to be equivalent to the material outlined above. The substance was referred to as metofluthrin and the minimum purity of the tested batches was given as 94.9% (based on total metofluthrin isomer content). However, the main 'RTZ' isomer (Epsilon-metofluthrin) was present in a concentration of >80.2% in all batches. In some cases, pure isomers (i.e., referred to as RTZ or RTE) were used. Where this is the case it is specified in the report.

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	Liquid	Section A3 Document IIIA	96.6 % (RTZ: 82.2 %)
Melting/freezing point	- 54°C	Section A3 Document IIIA	OECD guideline 102 using Differential Scanning Calorimetry (DSC) 99.8 % (RTZ: 86.6 %)
Boiling point	334°C	Section A3 Document IIIA	OECD guideline 103 using Differential Scanning Calorimetry (DSC) 99.8 % (RTZ: 86.6 %)
Relative density	1.21 at 20°C	Section A3 Document IIIA	OECD guideline 109 using Pycnometer 96.6 % (RTZ: 82.2 %)
Vapour pressure	$9.47x10^{-4}$ Pa at 20°C $1.96x10^{-3}$ Pa at 25 °C $6.85x10^{-3}$ Pa at 35°C $2.6x10^{-2}$ Pa at 45°C	Section A3 Document IIIA	OECD guideline 104 using Gas Saturation method 98.4 % (RTZ: 86.6 %)
Surface tension	Not applicable, water solubility < 1mg/l		
Water solubility	0.50 mg/l at 20 °C & pH 7.5 (98.5 % RTZ-isomer) 0.67 mg/l at 20 °C & pH 7.2 (99.2 % RTE-isomer)	Section A3 Document IIIA	OECD guideline 105 using Column elution method
Partition coefficient n-octanol/water	Log P_{ow} = 5.0 (98.7% RTZ isomer) Log P_{ow} = 5.0 (99.7 RTE isomer)	Section A3 Document IIIA	Directive 98/8/EC, Annex IIIa, section III, Point 1 using the Shake Flask method and chiral HPLC
Flash point	Flash point > 110 °C	Section A3 Document IIIA	ASTM D93-99a using Closed Cup method 96.6 % (RTZ: 82.2 %)
Flammability	Not flammable	Section A3 Document IIIA	Experience in handling and use shows that the substance does not spontaneously ignite in contact with air or water.
Explosive properties	Not Explosive	Section A3 Document IIIA	OPPTS 830.6316 96.6 % (RTZ: 82.2 %)

Self-ignition temperature	Spontaneous ignition temperature was 365 °C. No self ignition at temperatures up to the boiling point 334 °C	Section A3 Document IIIA	Directive 92/69/EEC, A15 95.4 % (RTZ: 85.6 %)
Oxidising properties	Not Oxidizing	Section A3 Document IIIA	OPPTS 830.6314 96.6 % (RTZ: 82.2 %)
Granulometry	Not applicable, substance is liquid		
Dissociation constant	No dissociation activity detected from pH 1 – 13 at 20 °C	Section A3 Document IIIA	OECD guideline 112 using Spectrophotometric method 99.8 % (RTZ: 86.6 %)
Viscosity	19.3 mm^2/s at 20 °C & 18.3 mm^2/s at 40 °C	Section A3 Document IIIA	OECD guideline 114 using Capillary Viscosity method (ISO 3104) 96.6 % (RTZ: 82.2 %)

All studies were conducted to GLP and were considered acceptable during the review of the active substance.

2 MANUFACTURE AND USES

2.1 Manufacture

Epsilon-metofluthrin is manufactured and formulated into biocidal products outside of the EU.

2.2 Identified uses

Epsilon-metofluthrin is used in biocidal products within the EU, including use as an insecticide for the control of flying insects (eg mosquitos).

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 9: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
Refer to table 8			

3.1 Physical Hazards

3.1.1 Summary and discussion of physico-chemical properties

Metofluthrin has a flash point of > 110 ^{o}C and is therefore not classified as a flammable liquid. Experience in handling and use indicates it is not pyrophoric and does not react with water to liberate flammable gases. Further, the spontaneous ignition temperature was determined to be 365 ^{o}C (EEC A15).

The substance does not exhibit explosive or oxidising properties and is not classified as such.

3.1.2 Conclusions on classification and labelling

Not classified – conclusive but not sufficient for classification.

4 HUMAN HEALTH HAZARD ASSESSMENT

A detailed summary of the available studies has been provided under the Biocidal Products Directive (98/8 EC). Document IIIA of the CAR (February 2010) is publically available on the ECHA website and appended to the technical dossier for further information. The key information relevant to determining a classification position is presented below.

Two batches have been used for the human health assessment. The purities reported in the study summaries refer to the total metofluthrin isomer content, but they contain 80.2% or 82.2% of the main 'RTZ' isomer (Epsilon-metofluthrin). These batches are considered to be representative of the technical material as outlined in section 1.2. In some cases the pure isomers (i.e. referred to as the RTZ or RTE isomers) were tested. Where this is the case it is specified in the report.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

The toxicokinetics of Metofluthrin have been well investigated in rats, following oral dosing of purified isomers (i.e., the RTZ and RTE isomers), in corn oil. An *in vivo* study in rats is also available investigating the dermal absorption of Metofluthrin, as supplied.

4.1.2 Human information

There is no human information available

4.1.3 Summary and discussion on toxicokinetics

When administered in corn oil (as the RTE or RTZ isomers), it is well absorbed (100%) following oral dosing. 100% uptake following inhalation exposure of neat Metofluthrin is anticipated, with 7-21% becoming systemically available following dermal exposure in rats. In vitro dermal absorption from a 1% Metofluthrin solution through human epidermis is only 8%. Metofluthrin is extensively metabolised following oral dosing. A wide range of Phase I reactions were identified, including C-oxidation, epoxidation and ester hydrolysis. The principal Phase II reactions are epoxide ring opening, glutathione conjugation and glucuronidation. Subsequent distribution of Metofluthrin metabolites is widespread. Elimination was rapid via both the urine and faeces. There were no marked gender-related differences in absorption, distribution, metabolism or excretion.

There is no information on any quantitative or qualitative differences that may exist between humans and rats or between the different isomers.

4.2 Acute toxicity

Table 10: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
Oral OECD TG 401 Rat, Sprague-Dawley, 5/sex/group 0, 1000, 1500, 2000 mg/kg Vehicle: none GLP	A single mortality (1/5 females)_was observed at the highest dose level of 2000 mg/kg. Clinical signs of toxicity (tremors, twitches, tachypnoea (females), excess salivation, prostrate, lost righting reflex, clonic convulsions, tonic extensor convulsions, and hyperpnoea) were observed at the top dose (2000 mg/kg)	LD_{50} > 2000 mg/kg	A6.1.1 Doc IIIA Kunimatsu (2002a)
Oral OECD TG 424 Rat, Sprague-Dawley, 10/sex/ group 0, 20, 50, 100 mg/kg Vehicle: corn oil GLP	Metofluthrin administered in a corn oil vehicle caused mortality in 3/20 animals (2/10M and 1/10 F) with 4 females sacrificed *in extremis* at 100 mg/kg (the highest dose tested). Clinical signs of toxicity were observed at the highest dose of 100 mg/kg including; Tremors in males and females. Tachypnoea in females. Excess salivation, prostrate, lost righting reflex, clonic convulsions in one dying male. Tonic extensor convulsions, lost righting reflex and hyperpnoea in another dying male.	A study conducted to investigate acute neurotoxicity	A6.9(.1)Doc IIIA) York, R.G. (2004a)
Oral (non standard) Mouse CD-1 strain 5/sex/group 12.5, 25, 50, 60 mg/kg Vehicle: corn oil GLP	1/5 males and 1/5 females of the 60 m/kg dose group died within 3 hours of dosing. In addition, 2/5 of the surviving males exhibited tremors at the 3 hours observation point. This had resolved 24 hours after dosing.	A sighting study for a mouse micronucleus test	A.6.6.4 Doc IIIA Odawara, K. (2002c)
Dermal OECD TG 402 Rat, Sprague-Dawley, 5/sex/group 2000 mg/kg GLP	No mortalities or clinical signs of toxicity.	LD50 >2000 mg/kg	A.6.1.2 Doc IIIA Kunimatsu (2002b)
Inhalation OECD TG 402 (nose only)	At 1 mg/l, a single female died (1/10 animals in the group) as a result of exposure; while at 2 mg/l all animals died (10/10) during or	LC50 1-2 mg/l	A.6.1.3 Doc IIIA Yoshihito, D.

Rat, Sprague-Dawley, 5/sex/group 0.5, 1, 2 mg/l, 4 hours Mist aerosol MMAD 3.36-3.86 µm GLP	immediately after exposure. Clinical signs of toxicity observed during exposure were confined to tail tremor, and observed in all exposure groups. Post-exposure, clinical signs of toxicity included tremor, hypersensitivity, ataxic gait, tiptoe gait and clonic convulsion, reported in all exposure groups. All clinical signs of toxicity had resolved one day after cessation of exposure. No further information on clinical signs, including incidence and severity were reported. There were no adverse changes observed in the respiratory tract of exposed animals following routine necropsy examination.		(2002)

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

In a standard acute toxicity study using neat liquid Metofluthrin, a single mortality (1/5 females) was observed at the highest dose of 2000 mg/kg.

In contrast, in an acute oral neurotoxicity study, Metofluthrin administered in a corn oil vehicle caused mortality in 3/20 animals (2M and 1F) with an additional 4 females sacrificed *in extremis* at 100 mg/kg (the highest dose tested). In the range finding study, Metofluthrin was administered to 5M and 5F rats at doses of 0, 20, 50 and 100 mg/kg. There were no deaths, but whole body twitches were observed in 2M and 4F at the 2 and 3 hour time points only. In a non-GLP compliant study subsequently conducted to evaluate the toxicity of Metofluthrin, 10 females were administered a single dose of 100 mg/kg Metofluthrin in corn oil, under the same conditions as the main acute neurotoxicity study. 1/10 females died 22 hours after dosing. This animal exhibited intermittent whole body twitches at approximately 4.5 hours after dosing, hyperactivity to sound at approximately 6 hours post dosing and continuous whole body tremors at approximately 6.5 hours after dosing. All other rats survived until terminal sacrifice but clinical observations in these animals included whole body tremors (6/10), whole body twitches (5/10) and hyperactivity to sound (6/10). These effects were seen from the 3.5 hour observation point and all surviving animals appeared normal the day after dosing.

Metofluthrin was also found to cause lethality when administered in a corn oil vehicle (2/10 at the highest dose of 60 mg/kg/day) in a sighting study for a mouse micronucleus test.

It is possible that the reason for the marked differences in acute oral toxicity observed between the two studies in rats is a consequence of Metofluthrin being administered neat in the standard study, and in corn oil in the neurotoxicity study. However, this is not considered to provide justification for using the TG 401 study to support a classification position.

4.2.1.2 Acute toxicity: inhalation

The 4-hour LC_{50} value was between 1 - 2 mg/l.

4.2.1.3 Acute toxicity: dermal

There was no evidence of systemic toxicity or mortalities at doses of up to 2000 mg/kg.

4.2.1.4 Acute toxicity: other routes

4.2.2 Human information

There is no relevant information available.

4.2.3 Summary and discussion of acute toxicity

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

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

It is possible there are vehicle dependent differences in toxicokinetics that are revealing an intrinsic hazardous property of Metofluthrin (acute oral toxicity). There do not appear to be clear methodological reasons to exclude findings from the single oral exposure studies with corn oil vehicle (as it is a standard vehicle). An LD_{50} value was not identified in the acute neurotoxicity study, but given the mortalities (7/20) at 100 mg/kg, classification for acute oral toxicity via the oral route is considered appropriate.

Please refer to section 4.2.1.2, and table 10 for information on acute inhalation toxicity.

4.2.4 Comparison with criteria

An LD_{50} value was not identified from the acute oral neurotoxicity study. However given the mortalities at 100 mg/kg/day (7/20) it is proposed to classify Metofluthrin with Acute Tox 3; H301 – Toxic if swallowed, considering the values of 50 mg/kg < ATE ≤ 300 mg/kg in Annex I of CLP.

An LC50 value of between 1-2 mg/L was observed in the acute inhalation study. Given the relevant values in Annex I of CLP for inhalation of dusts and mist (1 < ATE ≤ 5 mg/l) classification with Acute Tox 4, H332 – Harmful if swallowed is proposed.

4.2.5 Conclusions on classification and labelling

Acute Tox 3; H301 – Toxic if swallowed
Acute Tox 4; H332 – Harmful if inhaled

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

Table 11: Summary table of studies relevant for STOT SE

Method	Results	Remarks	Reference
Oral OECD TG 401 Rat, Sprague-Dawley, 5/sex/group 0, 1000, 1500, 2000 mg/kg Vehicle: none GLP	A single mortality was observed at the highest dose level of 2000 mg/kg. Clinical signs of toxicity (tremors, twitches, tachypnoea (females), excess salivation, prostrate, lost righting reflex, clonic convulsions, tonic extensor convulsions, and hyperpnoea) were only observed at the top dose (2000 mg/kg)	LD_{50} > 2000 mg/kg	A.6.1.1 (Doc IIIA) Kunimatsu (2002a)
Oral OECD TG 424 Rat, Sprague-Dawley, 10/sex/ group 0, 20, 50, 100 mg/kg Vehicle: corn oil GLP	Metofluthrin administered in a corn oil vehicle caused mortality in 3/20 animals with 4 animals sacrificed *in extremis* at 100 mg/kg (the highest dose tested). Clinical signs of toxicity were observed at the highest dose of 100 mg/kg including; Tremors in males and females. Tachypnoea in females. Excess salivation, prostrate, lost righting reflex, clonic convulsions in one dying male. Tonic extensor convulsions, lost righting reflex and hyperpnoea in another dying male.	A study conducted to investigate acute neurotoxicity	A.6.9(01) Doc IIIA York, R.G. (2004a)
Oral (non standard) Mouse CD-1 strain 5/sex/group 12.5, 25, 50, 60 mg/kg Vehicle: corn oil GLP	Metofluthrin caused mortalities (2/10) and tremor at the highest dose of 60 mg/kg,	A sighting study for a mouse micronucleus test	A.6.6.4 Doc IIIA Odawara, K. (2002c)

Inhalation OECD TG 402 (nose only) Rat, Sprague-Dawley, 5/sex/group 0.5, 1, 2 mg/l, 4 hours Mist aerosol MMAD 3.36-3.86 □m GLP	At 1 mg/l, a single female died (1/10 animals in the group) as a result of exposure; while at 2 mg/l all animals died (10/10) during or immediately after exposure. Clinical signs of toxicity observed during exposure were confined to tail tremor, and observed in all exposure groups. Post-exposure, clinical signs of toxicity included tremor, hypersensitivity, ataxic gait, tiptoe gait and clonic convulsion, reported in all exposure groups. All clinical signs of toxicity had resolved one day after cessation of exposure. No further information on clinical signs, including incidence and severity were reported. There were no adverse changes observed in the respiratory tract of exposed animals following routine necropsy examination.	LC50 1-2 mg/l	A.6.1.3 Doc IIIA Yoshihito, D. (2002)

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

Clinical signs of neurotoxicity were noted in a standard acute oral dosing study, at the highest dose of 2000 mg/kg, a dose that caused a single mortality. In one single exposure study (acute neurotoxicity) in which rats were dosed with Metofluthrin in corn oil, clinical signs of toxicity (tremors, twitches, tachypnoea (females), excess salivation, prostrate, lost righting reflex, clonic convulsions, tonic extensor convulsions, and hyperpnoea (males)) were only observed at the top dose of 100 mg/kg. At this dose, 2/10 males and 1/10 females were found dead and a further 4 females were sacrificed *in extremis*. In the range finding study, in which Metofluthrin was administered to 5M and 5F rats at doses of 0, 20, 50 and 100 mg/kg, there were no deaths. However, whole body twitches were observed in 2M and 4F at the 2 and 3 hour time points only. In a non-GLP compliant study subsequently conducted to evaluate the toxicity of Metofluthrin, 10 females were administered a single dose of 100 mg/kg Metofluthrin in corn oil, under the same conditions as the main acute neurotoxicity study. 1/10 females died 22 hours after dosing. This animal exhibited intermittent whole body twitches at approximately 4.5 hours after dosing, hyperactivity to sound at approximately 6 hours post dosing and continuous whole body tremors at approximately 6.5 hours after dosing. All other rats survived until terminal sacrifice and clinical observations in these animals included whole body tremors (6/10), whole body twitches (5/10) and hyperactivity to sound (6/10). These effects were seen from the 3.5 hour observation point and all surviving animals appeared normal the day after dosing.

Tremors were noted in mice administered Metofluthrin in corn oil at a dose of 60 mg/kg, a dose that caused mortalities (2/10).

Overall, Metofluthrin induced clinical signs of neurotoxicity, but only at doses that caused mortalities.

In a standard acute inhalation study clinical signs of toxicity were observed during exposure in all exposure groups but were confined to tail tremor. Post-exposure, clinical signs of toxicity included tremor, hypersensitivity, ataxic gait, tiptoe gait and clonic convulsion and were reported in all exposure groups to varying degrees. All clinical signs of toxicity had resolved one day after cessation of exposure.

4.3.2 Comparison with criteria

Signs of neurotoxicity were observed below the guidance value for classification with STOT-SE 1 (i.e. at < 300 mg/kg via oral exposure and <1 mg/L via inhalation). As such, a simple argument for classification with STOT-SE 1 could be made. However, as the effects in the acute neurotoxicity study occurred at doses causing lethality and given the mortalities at 1 and 2 mg/L in the acute inhalation study, it is proposed to classify for acute toxicity as outlined in section 4.2 of this report instead.

4.3.3 Conclusions on classification and labelling

Not classified – Conclusive but not sufficient for classification

4.4 Irritation

4.4.1 Skin irritation

Table 12: Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
Rabbit (New Zealand White) 3 Males OECD TG 404 GLP	Mean scores over 24-72 hours in 3 rabbits Erythema: 0.6, 0.6, 0.3 Oedema: 0, 0, 0	The observed changes resolved within 7-days	A.6.1.4 Doc IIIA Nakamura, Y. (2001a)

4.4.1.1 Non-human information

The skin irritation potential of Metofluthrin has been well investigated in a standard study in rabbits. The findings are reported in table 12 above.

4.4.1.2 Human information

There is no information available

4.4.1.3 Summary and discussion of skin irritation

Please see above.

4.4.1.4 Comparison with criteria

Metofluthrin caused mild transient erythema but the mean scores do not meet the criteria for classification as a skin irritant, when compared to the criteria for classification (i.e., all scores are < 2.3 for erythema and oedema in all animals).

4.4.1.5 Conclusions on classification and labelling

Not classified – Conclusive but not sufficient for classification.

4.4.2 Eye irritation

Table 13: Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
Rabbit (New Zealand White) 3 Male OECD TG 405 GLP	Cornea: 0, 0, 0 Iris: 0, 0, 0 Conjunctival redness: 0, 0, 0 Conjunctival chemosis: 0, 0, 0	Mean scores over 24-72 hours in 3 rabbits	A.6.1.4 Doc IIIA Nakamura, Y. (2001a)

4.4.2.1 Non-human information

The eye irritation potential of Metofluthrin has been well investigated in a standard study in rabbits; the findings are reported in table 13 above.

4.4.2.2 Human information

There is no information available

4.4.2.3 Summary and discussion of eye irritation

Please see above.

4.4.2.4 Comparison with criteria

The mean scores do not meet the criteria for classification as an eye irritant (i.e. all scores were 0), when compared to the criteria.

4.4.2.5 Conclusions on classification and labelling

Not classified – Conclusive but not sufficient for classification

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

In single and repeated inhalation exposure studies in rats, no clinical signs of toxicity or histopathological changes consistent with respiratory tract irritation were observed.

4.4.3.2 Human information

No symptoms of respiratory tract irritation have been reported following routine health surveillance of workers involved in Metofluthrin manufacture although it is noted that there is limited information available.

4.4.3.3 Summary and discussion of respiratory tract irritation

Please see sections 4.4.3.1 and 4.3.3.2

4.4.3.4 Comparison with criteria

No symptoms of respiratory tract irritation were observed in potentially exposed humans. No evidence of respiratory tract irritation was observed in relevant studies in experimental animals. Therefore, it can be concluded that Metofluthrin does not meet the criteria for classification.

4.4.3.5 Conclusions on classification and labelling

Not classified – Conclusive but not sufficient for classification

4.5 Corrosivity

As the substance was found to be a very mild irritant in a standard animal study, discussion of skin corrosivity is not required.

4.6 Sensitisation

4.6.1 Skin sensitisation

Table 14: Summary table of relevant skin sensitisation studies

Method	Doses	No. sensitised/total no	Result	Reference
Guinea Pig (Hartley) OECD TG 406 Guinea Pig Maximisation test 20 test and 10 controls	Induction: Intradermal: 5% in corn oil Topical: 100% Challenge: 25% in acetone Doses selected from a preliminary study. Slight erythema was observed at an intradermal dose of 0.5% and above, no skin reactions were observed at a topical dose of 25%.	Test: 0/20 Negative Control (corn oil): 0/10 Positive control (HCA): 5/5 (100%) HCA control: 0/5.	Negative	A.6.1.5 Doc IIIA Nakamura, Y. (2002b)

4.6.1.1 Non-human information

Metofluthrin tested negative in a standard maximisation test.

4.6.1.2 Human information

No incidences of skin sensitisation have been reported following routine health surveillance, although it is noted that there is limited information available.

4.6.1.3 Summary and discussion of skin sensitisation

Please see table 14, and sections 4.6.1.1 and 4.6.1.2.

4.6.1.4 Comparison with criteria

Metofluthrin does not meet the criteria for classification.

4.6.1.5 Conclusions on classification and labelling

Not classified – conclusive but not sufficient for classification.

4.6.2 Respiratory sensitisation

4.6.2.1 Non-human information

This potential of Metofluthrin to cause respiratory sensitisation was not investigated directly. However, given that Metofluthrin does meet the criteria for classification for skin sensitisation and the sub-acute inhalation study gave no indication of respiratory sensitisation, Metofluthrin is considered unlikely to be a respiratory sensitiser. Therefore no classification is proposed.

4.6.2.2 Human information

No data are available.

4.6.2.3 Summary and discussion of respiratory sensitisation

See section 4.6.2.1.

4.6.2.4 Comparison with criteria

See section 4.6.2.1.

4.6.2.5 Conclusions on classification and labelling

Not classified – Data lacking

4.7 Repeated dose toxicity

The repeated dose toxicity of Metofluthrin has been investigated by the oral route in rats (dietary studies of between 28 days to 2 years duration), mice (dietary studies of 13 weeks and 2 years duration) and dogs (capsule dosing studies of 13 weeks and 1 year duration); in addition, a specific 90 day dietary study in rats has been conducted to investigate its neurotoxic potential. The repeated dermal toxicity of Metofluthrin has been investigated in a 13 week study in rats and the repeated inhalation toxicity of Metofluthrin has been investigated in a 28 day study in rats.

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Studies in rats

Table 15: Summary table of relevant repeated dose toxicity studies

Method	Results	Reference
EC B7 28-days , diet Rat, Wistar, 12/sex/ group, plus 0, 300, 1000 and 3000 ppm ad lib Estimated to be approximately 0, 29, 96 and 285 mg/kg/day in males and 0, 29, 95 and 273 mg/kg/day in females GLP Guideline value for classification: ≤ 300 mg/kg bw/d	**3000 ppm (285/273 mg/kg/day m/f):** Tremor (12/18 males; 15/18 females) Increased plasma cholesterol (70 % males, 43 % females) Increased plasma phospholipids (57 % males, 24 % females). Increased liver weight (20 %, males and females). Increased spleen weight (17 % females) Decreased ventral prostate weight (25 % males)Slight-mild hepatocellular hypertrophy (10/12 males, 7/11 females) All changes resolved during the 14-day recovery period **1000 ppm (96/95 mg/kg/day m/f):** Increased plasma cholesterol (28 % males) Increased plasma phospholipids (21 % males) Slight-mild hepatocyte hypertrophy (3/12 males, 1/12females) ***300 ppm (29 mg/kg/day m/f):*** No treatment-related changes reported	A.6.3.1 doc IIIA Kunimatsu (2002c)
OECD TG 408 90-days , diet Rat, Wistar, 12/sex/ group, 0, 100, 300, 1000 and 2500 ppm Estimated to be approximately 0, 6.9, 21, 70 and 184 mg/kg/day in males and 0, 7.5, 22, 73 and 186 mg/kg/day in females This study also included a functional observational battery (FOB). GLP Guideline value for classification: ≤100 mg/kg bw/d	***2500 ppm (184/186 mg/kg/day m/f):*** Increased plasma cholesterol (37 % males, 33 % females), Increased plasma phospholipids (31 % males, 25 % females) Increased urinary leucocytes (350 % males)Increased liver weight (22 % males, 16 % females), Hepatocellular basophilia (12/12 males, 4/12 females) Hepatocellular hypertrophy (12/12 males, 5/12 females) ***1000 ppm (70 and 73 mg/kg/day m/f):*** Increased plasma cholesterol (26 % males); Increased plasma phospholipids (19 % males); Increased urinary leucocytes (150 % males); Increased liver weight (17 % males); Hepatocellular basophilia (12/12 males, 1/12 females) Hepatocellular hypertrophy (10/12 males, 1/12 females) ***300 ppm (21/22 mg/kg/da m/f):*** Hepatocellular basophilia (5/12 males); Hepatocellular hypertrophy (5/12 males) ***100 ppm (6.9/7.5 mg/kg/day m/f):*** No treatment-related changes reported No toxicologically significant changes were noted in the FOB, at any dose level	A.6.4.1(01) doc IIIA Sommer *et al* (2003)

OECD TG 424 (neurotoxicity) 90-days , diet Rat, Sprague Dawley 12/sex/ group, 0, 300, 1000 and 3000 ppm Estimated to be approximately: 0, 18, 60 or 179 mg/kg/day in males, and 0, 21, 69 or 206 mg/kg/day in females GLP Guideline value for classification: ≤100 mg/kg bw/d	***3000 ppm (179/206 mg/kg/day m/f):*** Treatment-related mortality in a single female; Decreased bodyweight gain (15 % males, 25 % females); Tremor (1/12 males, 4/12 females); Twitch (3/12 females); No evidence of pathology or permanent neurotoxic change ***300 ppm (18/21 mg/kg/day m/f) and 1000 ppm (60/69 mg/kg/day m/f):*** No treatment-related changes reported. No toxicologically significant changes were noted in the FOB, at any dose level	A6.9(02) Doc IIIA) York, R.G. (2004b)
26-weeks, diet, non standard study Rat, Sprague Dawley, 12/sex/ group, 0, 100, 300, 1000 and 3000 ppm ad lib Estimated to be approximately: 0, 5.3, 16, 54 and 165 mg/kg/day in males and 0, 6.4, 19, 69, and 191mg/kg/day in females There is no EU guideline or requirement for a 26-week study in rats. The study fulfils EC Guideline B26: Sub-chronic oral toxicity test. Repeated dose 90-day toxicity study GLP Guideline value of ≤ 50 mg/kg/day is considered for classification: calculated from the value defined for the rat 90 day study.	***3000 ppm (165/191 mg/kg/day):*** Tremor in all animals during week 1, declining rapidly until no tremors observed in week 4 Increased plasma cholesterol (62 % males); Increased plasma phospholipids (77 % males); Increased plasma bilirubin (78 % males); Decreased plasma bilirubin (26 % females) Increased liver weight (20 % males); Dark liver (9/12 males, 7/12 females); Enlarged liver (7/12 males, 7/12 females); Hepatocellular hypertrophy (12/12 males, 10/12 females); Hepatocellular steatosis (9/12 males) ***1000 ppm (54/69 mg/kg/day):*** Increased plasma cholesterol (35 % males); Increased plasma phospholipids (37 % males) Dark liver (5/12 males) Hepatocellular hypertrophy (9/12 males, 4/12 females) ***100 ppm (5.3/6.4 mg/kg/day m/f and 300 ppm (16/19 mg/kg/day m/f):*** No treatment-related changes reported	A6.5(01) Doc IIIA Kunimatsu, (2002d)
OECD TG 453 2-years , diet Rat, Wistar, 50/sex/ group, plus	***1800 ppm (78/96 mg/kg/day m/f):*** Decreased bodyweight gain (wk 78) (12 % males, 19 % females) Decreased final bodyweight (12 % males, 15 %	A6.5(03) Doc IIIA Schmid *et al*

20/sex/group for a planned interim sacrifice 0, 20, 200, 900 and 1800 ppm Estimated to be approximately: 0, 0.8, 8, 38 and 78 mg/kg/d in males and 0, 1.0, 10, 47 and 96 mg/kg/d in females This study also included a functional observational battery (FOB). Guideline value of ≤ 12 mg/kg/day is considered for classification: calculated from the value defined for the rat 90 day study.	females) Increased plasma cholesterol (wk 53- 37 % males); Increased plasma triglycerides (wk 53 - 49 % males); Increased plasma phospholipids (wk 53 - 41 % males); Increased plasma gamma glutamyl tramsferase (GGT) (wk 53 - males 2.46 U/l; 0 U/l in control males) Liver – hepatocellular hypertrophy (13/50 males); clear cell foci (32/50 females) eosinophilic foci (10/50 males);; mixed cell foci (12/50 females) Kidney – A statistically significant increases in the incidence of lipofuscin deposition in males (12/50 at 78 mg/kg/d compared to 3/50 in controls) and females (40/50 at 96 mg/kg/d compared to 21/50 in controls); A statistically significant increases in the incidence of tubular casts (48/50 at 96 mg/kg/d compared to 40/50 in controls) and interstitial fibrosis (10/50 at 96 mg/kg/d compared to 3/50 in controls) in top dose females; A statistically significant increase in the incidence of tubular vacuolation in top dose males (8/50 at 78 mg/kg/d compared to 0/50 in controls). ***900 ppm (38/47 mg/kg/day m/f):*** Increased plasma cholesterol (wk 53) (24 % males); Increased plasma triglycerides (wk 53) (62 % males); Increased plasma phospholipids (wk 53) (26 % males) Liver – hepatocellular hypertrophy (12/50 females) and mixed cell foci (9/50 males) ***20 ppm 0.8/1.0 mg/kg/day and 200 ppm (8/10 mg/kg/day m/f):*** No treatment-related changes were observed	(2005c)

Studies in mice

Table: 16 Summary table of relevant repeated dose toxicity studies

Method	Results	Reference
EC B26 90-days , diet Mouse, CD-1, 0, 100, 1500, 2500 and 3500 ppm ad lib Estimated to be approximately: 0, 14, 209, 357 and 487 mg/kg/day in males and 0, 17, 252, 439 and 587 mg/kg/day in females GLP	***3500 ppm 487/587 mg/kg/day m/f):*** Increased plasma cholesterol (55 % females); Increased plasma phospholipids (50 % females); Increased liver weight (18 % males, 19 % females) Hepatocellular hypertrophy (7/12 males, 9/12 females); Hepatocellular degeneration/necrosis (3/12 males, 3/12 females) ***2500 ppm (357/439 mg/kg/day m/f):*** Increased plasma cholesterol (45 % females); Decreased WBC (50 % males) Increased liver weight (22 % males)	A6.4.1(03) Doc IIIA Sommer *et al* (2004)

Guideline value of ≤ 100 mg/kg/day is considered for classification: based on the value defined for the rat 90 day study.	Hepatocellular hypertrophy (7/12 males, 11/12 females) ***1500 ppm (252/209 mg/kg/day m/f):*** Increased liver weight (19 % males) Hepatocellular hypertrophy (4/12 males, 8/12 females) ***100 ppm (14/17 mg/kg/day m/f):*** No treatment-related changes reported	
OECD TG 451 78 weeks , diet Mouse CD-1, 52/sex/ group, plus 12/sex/group for a planned interim sacrifice 0, 100, 1000 and 2500/1750 ppm Estimated to be approximately: 0, 12, 116, and 209 mg/kg/day in males and 0, 15 155 and 277 mg/kg/day in females GLP Guideline value of ≤ 12 mg/kg/day is considered for classification: calculated from the value defined for the rat 90 day study.	***2500 ppm):*** Mortality in 12/64 males and 4/64 females. Top dose reduced to 1750 ppm from the second week of the study. ***2500/1750 ppm (209/277 mg/kg/day m/f :*** Hepatocellular hypertrophy (16/52 females) ***1000 ppm (116/155 mg/kg/day m/f):*** Hepatocellular hypertrophy (17/52 females) ***100 ppm (12/15 mg/kg/day m/f):*** No treatment-related changes reported	A6.5(04) Doc IIIA Schmid *et al* (2005d)

Studies in dogs

Table 17: Summary table of relevant repeated dose toxicity studies

Method	Results	Reference
EC B27 90-days , capsule Beagle dog, 4/sex/dose, Plus 2/sex in control and high dose for 6 week recovery period 0, 10, 30 and 100 mg/kg/day, 7 days per week Guideline value of ≤ 100 mg/kg/day is considered for classification: taken from the value defined for the rat 90 day study.	***100 mg/kg/day*** Increased incidence of tremor (5/6 males, 5/6 females), 2 – 6 h, post-administration Increased incidence of vomiting post-administration ***30 mg/kg/day:*** Increased incidence of vomiting post-administration ***10 mg/kg/day:*** No treatment-related changes reported	A6.4.1(02) Doc IIIA Uchida (2002)
OECD TG 452 1-year, capsule Beagle dog, 4/sex/dose, 0, 10, 30 and 100 mg/kg/day, 7 days per week Guideline value of ≤ 24 mg/kg/day is considered for classification: calculated from the value defined for the rat 90 day study.	***100 mg/kg/day:*** Increased incidence of tremor (4/4 males, 3/4 females), 2 – 6 h, post-administration Increased incidence of vomiting post-administration **30 mg/kg/day**: Increased incidence of tremor (4/4 males, 3/4 females) Increased incidence of vomiting post-administration Increased plasma triglycerides (112 % females) ***10 mg/kg/day:*** No treatment-related changes reported	A6.5(02) Doc IIIA Uchida (2004)

The most prominent findings, from the oral repeated dose studies were neurotoxicity in rats and dogs, hepatotoxicity in rats and mice and indications of nephrotoxicity in rats.

Neurotoxicity

A 90 day dietary study in rats is available, specifically designed to investigate neurotoxicity. At the top dose level in this study (179 and 206 mg/kg/day in males and females, respectively), only one intercurrent mortality was reported; there was a statistically significant decrease in bodyweight gain in males and females (15 % and 25 %, respectively); and an increased incidence of tremor (1/12 males, 4/12 females) and twitches in females (3/12). Detailed histopathological examination of the peripheral and CNS tissues found no treatment-related changes. No positive findings were observed in any of the FOB (Functional Observational Battery) investigations conducted.

In a 28 day dietary study in rats, an increased incidence of tremor was reported in top dose animals only (12/18 males and 15/18 females administered 285 and 273 mg/kg/day, respectively), which

resolved by day 14. No tremors or other clinical signs of neurotoxicity were observed at dose levels of 96 mg/kg/day and below. In a 26 week dietary study in rats, an increased incidence of tremor was reported in all top dose animals (males 165 mg/kg/day, females 191 mg/kg/day) during the first week of administration, which resolved by week 4 of the study. No tremors were reported at dose levels of 69 mg/kg/day and below. It is noted that no increased incidences of tremor are reported in the standard 90 day dietary study in rats at dose levels of up to 186 mg/kg /day or in the lifetime study in rats at dose levels of up to 96 mg/kg /day.

In dogs, evidence of the neurotoxic potential of Metofluthrin was reported in capsule studies of 90 days and 1-year duration. An increased incidence of tremor was reported at the top dose level of 100 mg/kg/day in both the 90 day (5/6 males, 5/6 females) and 1 year (4/4 males, 3/4 females) studies. In addition, an increased incidence of tremor was also reported in the 1 year study at a dose level of 30 mg/kg/day (4/4 males, 1/4 females). The tremors usually occurred 2 – 6 h following administration. In both studies, an increased incidence of vomiting post-administration was noted in both sexes at dose levels of 30 and 100 mg/kg/day.

Whilst neurotoxic effects were observed below the guidance values for classification, they are considered to be the result of the acute exposure.

Hepatotoxicity

Hepatotoxicity in both rats and mice has been observed in standard studies following exposure to Metofluthrin in the diet. This is manifested as: increased levels of plasma cholesterol, phospholipids and gamma glutamyl transferase activity and histopathological changes such as hepatocellular hypertrophy and basophilic, eosinophillic and clear cell foci, and increased liver weight. Steatosis was only observed in rats administered Metofluthrin at doses of 165-191 mg/kg/day for 26-weeks, the highest dose tested.

In these studies the most sensitive marker of hepatotoxicity is considered to be hepatocellular hypertrophy. Increases in the incidence of hepatocellular hypertrophy were observed in the rat following exposure for 28 days, 90 days, 26 weeks and 2 years at dose levels of 95 mg/kg/day, 21 mg/kg/day, 54 mg/kg/day and 47 mg/kg/day and above, respectively. The mouse was less sensitive to the hepatotoxicity of Metofluthrin, with statistically significant increases in the incidence of hepatocellular hypertrophy being reported at dose levels of 209 mg/kg/day and 155 mg/kg/day and above following dietary exposure for 90 days and 78 weeks, respectively.

Mechanistic investigations in rats (see Section 4.12.1.1) show that dietary administration of Metofluthrin can induce hepatic xenobiotic metabolising enzymes, which is a well-established cause of hepatocyte hypertrophy. The profile of enzyme induction following Metofluthrin exposure (in particular CYP 2B1/2) is similar to that produced by phenobarbital. Hepatocyte hypertrophy secondary to phenobarbital-like enzyme induction may be regarded as an adaptive response. However, in this instance, it is also associated with statistically significant increases in plasma of cholesterol and phospholipids levels, which are likely to be an expression of liver toxicity.

Nephrotoxicity

Indications of nephrotoxicity were observed in the lifetime dietary study in rats, however, it should be noted that in general the findings showed only weak dose response relationships. The nephrotoxicity was considered treatment-related in top dose group animals only. These changes did not lead to any physiological disturbance. Overall, nephrotoxicity occurred in a single species at high doses in a lifetime study only.

4.7.1.2 Repeated dose toxicity: inhalation

Table 18: Summary table of relevant repeated dose toxicity studies

Method	Results	Reference
OECD TG 412 28-days Rat, Sprague-Dawley, 10/sex/group 0, 0.01, 0.05, 0.1, 0.2 mg/l 4h/day, 7 days/wk Aerosol administration Guideline value of ≤ 0.6 mg/L/day is considered for classification: based on the value defined for the rat 90 day study.	***0.2 mg/l:*** Treatment-related mortality (7 /10 males, 3/10 females). The 3 females died on days 3,4 and 5 respectively. The male deaths were distributed throughout the study with 3 on day 4 and 1 on each of days 9, 19, 25 and 27 respectively. Tremors, observed during or immediately after exposure (5/10 males, 5/10 females) ***0.01/0.05/0.1mg/l:*** No treatment-related changes reported	A6.3.3 Doc IIIA Deguchi (2002) (

Following repeated inhalation exposure of rats to Metofluthrin at concentrations of 0.01 - 0.2 mg/l, 4 h/d, 7 day/wk for 28 days, no treatment-related effects were observed at exposures of up to 0.1 mg/l. At the highest concentration of 0.2 mg/l, mortalities occurred throughout the study period; and tremor suggestive of neurotoxicity was also observed. No histopathological changes were observed locally or systemically.

4.7.1.3 Repeated dose toxicity: dermal

Table 19: Summary table of relevant repeated dose toxicity studies

Method	Results	Reference
OECD TG 411 13-weeks Rat, Sprague-Dawley, 10/sex/group 0, 30, 100, 300 and 1000 mg/kg/day, 6 hours/day, 7 days/wk Guideline value of ≤200 mg/kg/day is considered for classification.	***1000 mg/kg/day:*** Treatment-related mortality (2/12 females) Tremor in a single female prior to death Slight squamous cell hyperplasia at application site (5/10 females) ***30/100/300 mg/kg/day:*** No treatment-related changes reported	A6.4.2(01) Deguchi (2002)

Following repeated dermal application of between 0 - 1000 mg/kg/day Metofluthrin to rats for 13 weeks, no toxicologically significant, treatment-related changes were observed at doses of up to 300

mg/kg/day. At the highest dose of 1000 mg/kg/day, 2/12 female animals died within 3 days of the start of the study; tremor was noted in one of these females prior to death. A single mortality was reported in males of the top dose group, but this was not considered treatment related. The only histopathological change was an increased incidence of squamous cell hyperplasia in females at 1000 mg/kg/day. No toxicologically significant local changes were observed in males.

4.7.1.4 Repeated dose toxicity: other routes

4.7.1.5 Human information

There is no information available

4.7.1.6 Other relevant information

4.7.1.7 Summary and discussion of repeated dose toxicity

See previous sections (4.7.1.1, 4.4.1.2 and 4.7.1.3)

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

A substance is classified for STOT-RE in accordance with CLP when specific target organ toxicity arises from repeated exposure to concentrations at or below specified levels.

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE

See previous sections (4.7.1.1, 4.4.1.2 and 4.7.1.3)

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

Oral

An increased incidence of hepatocellular hypertrophy was observed in the rat following repeated oral exposure to Metofluthrin. In a standard 90-day dietary study, hepatocyte hypertrophy accompanied by hepatocyte basophilia was observed at doses of 21-22 mg/kg/day and above. Marked liver weight increases (around 20%) were only observed at doses of 70-73 mg/kg/day and above. There was no evidence of functional disturbance at any dose level. Overall, hepatocyte hypertrophy in the absence of marked liver weight increases or perturbation of liver function does not support classification for repeated dose toxicity. No classification is proposed.

Dermal

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

Inhalation

The classification cut-off for STOT-RE category 2 for effects in rat 90-day inhalation studies is 0.02-0.2 mg/l/6h day. This scales to 0.06-0.6 mg/l/6 hr day for an equivalent 28-day study.

Following repeated inhalation exposure of rats for 28 days to a Metofluthrin aerosol, mortality and tremors were observed at a dose 0.2 mg/l/4hr day, the highest concentration tested. As mortalities occurred throughout this study and are not considered to be a manifestation of the acute inhalation toxicity of Metofluthrin, classification with STOT-RE Category 2 is proposed.

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

STOT-RE 2; H373 – May cause damage to organs through prolonged or repeated exposure (inhalation).

4.9 Germ cell mutagenicity (Mutagenicity)

4.9.1 Non-human information

4.9.1.1 In vitro data

Table 20: Summary of relevant *in vitro* data

Test system/ Method/Guideline	Organism/Strain	Concs. tested	Result		Remarks (e.g. cytotoxicity)	Reference
			+ S9	- S9		
Bacterial Reverse Mutation Test OECD TG 471 GLP	*S.typhimurium*: TA 1535, TA 1537, TA 98, TA 100, E. coli: WP2 uvrA	156 – 5000 µg/plate	-ve	-ve	No evidence of cytotoxicity up to the highest dose tested.	A6.6.1 Doc IIIA Kit S. (2002)
Chromosomal aberration test OECD TG 473 GLP	Chinese hamster lung cells	50 – 250 µg/mL	-ve	-ve	Marked cytotoxicity to growth rate of 50% or lower was seen at the top doses evaluated in any treatment conditions.	A6.6.2 Doc IIIA Odawara, K. (2002a)
In vitro gene mutation assay (HPRT) in mammalian cells OECD TG 476 EC B17 GLP	Chinese hamster ovary cells (CHO-K1 BH4)	14.07 – 675 µg/mL	-ve	-ve	Clear dose related cytotoxicity was observed in the absence of metabolic activation in Experiment 1. In other experimental conditions, no marked cytotoxicity was seen but precipitates forming oily film on the surface of the media were observed.	A6.6.3 doc IIIA Durward, R. (2005)

4.9.1.2 *In vivo* data

Table 21: Summary of relevant *in vivo* data

Test method/ Guideline	Sampling times	Dose levels	Results	Remarks	Reference
Micronucleus Test Mouse, CD-1, 5 males/ group OECD TG 474 EC B12 GLP	24 and 48 hours	0, 12.5, 25, 50 mg/kg	Negative at all dose levels at both time points	No change in NCE/PCE ratio. Mortalities at 60 mg/kg in sighting study	A6.6.4(Doc IIIA) Odawara, K. (2002b)

4.9.2 Human information

There is no human information available

4.9.3 Other relevant information

4.9.4 Summary and discussion of Mutagenicity

The genotoxicity of Metofluthrin has been investigated, both *in vitro* (bacterial gene mutation, mammalian cell gene mutation and chromosomal aberration) and *in vivo* (mouse micronucleus), and gave negative results in all tests. Although no reduction in PCE/NCE ratio was observed, there are data from toxicokinetic studies indicating that following oral dosing, Metofluthrin is distributed to the bone marrow. Overall, it can be concluded that Metofluthrin does not have genotoxic potential,

4.9.5 Comparison with criteria

As Metofluthrin tested negative *in vitro* and *in vivo*, and there are no human data available, classification for genotoxicity is not justified.

4.9.6 Conclusions on classification and labelling

Not Classified – Conclusive but not sufficient for classification.

4.10 Carcinogenicity

The carcinogenic potential of Metofluthrin has been investigated by the oral route, in dietary studies of 2 years duration in rats and mice.

Table 22: Summary table of relevant carcinogenicity studies

Method	Results/Remarks	Reference
OECD TG 453 2-years , diet Rat, Wistar, 50/sex/ group, plus 20/sex/group for a planned interim sacrifice 0, 20, 200, 900 and 1800 ppm Estimated to be approximately: 0, 0.8, 8, 38 and 78 mg/kg/day in males and 0, 1.0, 10, 47 and 96 mg/kg/day in females This study also included a functional observational battery	**<u>Non-neoplastic findings</u>:** ***1800 ppm (78/96 mg/kg/day m/f):*** Decreased bodyweight gain (wk 78) (12 % males, 19 % females) Decreased final bodyweight (12 % males, 15 % females) Increased plasma cholesterol (wk 53- 37 % males); Increased plasma triglycerides (wk 53 - 49 % males); Increased plasma phospholipids (wk 53 - 41 % males); Increased plasma gamma glutamyl tramsferase (GGT) (wk 53 - males 2.46 U/l; 0 U/l in control males) Liver – hepatocellular hypertrophy (13/50 males); clear cell foci (32/50 females) eosinophilic foci (10/50 males);; mixed cell foci (12/50 females) Kidney – A statistically significant increases in the incidence of lipofuscin deposition in males (12/50 at 78 mg/kg/d compared to 3/50 in controls) and females (40/50 at 96 mg/kg/d compared to 21/50 in controls); A statistically significant increases in the incidence of tubular casts (48/50 at 96 mg/kg/d compared to 40/50 in controls) and interstitial fibrosis (10/50 at 96 mg/kg/d compared to 3/50 in controls) in top dose females; A statistically significant increase in the incidence of tubular vacuolation in top dose males (8/50 at 78 mg/kg/d compared to 0/50 in controls). ***900 ppm (38/47 mg/kg/day m/f):*** Increased plasma cholesterol (wk 53) (24 % males); Increased plasma triglycerides (wk 53) (62 % males); Increased plasma phospholipids (wk 53) (26 % males) Liver – hepatocellular hypertrophy (12/50 females) and mixed cell foci (9/50 males) ***20 ppm 0.8/1.0 mg/kg/day and 200 ppm (8/10 mg/kg/day m/f):*** No treatment-related changes were observed **<u>Neoplastic Findings</u>:** <u>Hepatocellular Carcinoma</u>: Males; 0, 0, 0, 6, and 16% at 0, 0.8, 8.2, 38 and 78 mg/kg/day, Females; 0, 4, 2, 4 and 14 %, at 0, 1.0, 10, 47 and 96 mg/kg/day <u>Hepatocellular adenoma</u>: Males; 2, 2, 6, 10, and 12 % at 0, 0.8, 8.2, 38 and 78 mg/kg/day Females; 2, 2, 0, 6 and 14 % in females at 0, 1.0, 10, 47 and 96 mg/kg/day	A6.5(03) Doc IIIA Schmid *et al* (2005c)

OECD TG 451 78 weeks , diet Mouse CD-1, 52/sex/ group, plus 12/sex/group for a planned interim sacrifice 0, 100, 1000 and 2500/1750 ppm Estimated to be approximately: 0, 12, 116, and 209 mg/kg/day in males and 0, 15, 155 and 277 mg/kg/day in females	**<u>Non-neoplastic findings:</u>** ***2500 ppm):*** Mortality in 12/64 males and 4/64 females. Top dose reduced to 1750 ppm from the second week of the study. ***2500/1750 ppm (209/277 mg/kg/day m/f :*** Hepatocellular hypertrophy (16/52 females) ***1000 ppm (116/155 mg/kg/day m/f):*** Hepatocellular hypertrophy (17/52 females) ***100 ppm (12/15 mg/kg/day m/f):*** No treatment-related changes reported **Neoplastic Findings:** No treatment-related change in tumour incidence	A6.5(04) Doc IIIA Schmid *et al* (2005d)

4.10.1 Non-human information

There is no information on the carcinogenic potential of Metofluthrin in humans.

4.10.1.1 Carcinogenicity: oral

An increased incidence of liver carcinomas and adenomas was observed in rats. The incidence of liver carcinomas was statistically significantly increased at the top dose in both males and females when compared to concurrent controls (0, 0, 0, 6, and 16 % in males at 0, 0.8, 8.2, 38 and 78 mg/kg/day, respectively and 0, 4, 2, 4 and 14 % in females, at 0, 1.0, 10, 47 and 96 mg/kg/day, respectively). The contemporary historical control incidence for rat liver carcinomas in the test laboratory is 0 - 2 % in both males and females. Thus, the observed incidence of liver carcinomas in males exceeded both the concurrent and historical control incidence at doses of 38 mg/kg/day and above. In females, the incidence of carcinomas was increased at all dose levels, markedly so at 96 mg/kg/day, the highest dose tested.

The incidence of liver adenomas was statistically significantly increased at the top dose in both males and females when compared to concurrent controls (2, 2, 6, 10, and 12 % in males at 0, 0.8, 8.2, 38 and 78 mg/kg/day, respectively and 2, 2, 0, 6 and 14 % in females at 0, 1.0, 10, 47 and 96 mg/kg/day). The contemporary historical control incidence for rat liver adenomas in the test laboratory is 0 - 8 % and 0 - 10 % in males and females, respectively. In this study, the incidence of liver adenomas clearly exceeds the historical control incidence at the top two doses in males, while in females, the incidences are within the historical control range.

No treatment-related increase in tumour incidence was observed in mice at doses of up to 209 mg/kg/day in males and 277 mg/kg/day in females (the highest doses tested) in a lifetime study.

4.10.1.2 Carcinogenicity: inhalation

There are no data available

4.10.1.3 Carcinogenicity: dermal

There are no data available

4.10.2 Human Information

4.10.3 Other relevant information

The UK Competent Authority has had extensive discussions with the applicant regarding the mode of action (MOA) for the liver tumours observed in rats treated with Metofluthrin. The applicant provided a document (please refer to Annex I) outlining their position and the mechanistic data available to justify the proposed MOA that Metofluthrin induces liver tumours in rats via interaction with the constitutive androstane receptor (CAR). The document also proposes that the mechanism described is not relevant for human health. A number of *in vivo* and *in vitro* non-guideline mechanistic studies have been carried out by industry to identify a MOA and support their contention that rat liver tumours are not relevant for human health (summarised below).

***In vitro* studies**

Three studies are available, one investigating enzyme induction and two investigating both enzyme induction and cell proliferation.

Test system	Concs. tested	Result	Remarks (e.g. cytotoxicity)
Deguchi Y *et al* (2009) Study to investigate gene expression specific to CAR activation Primary cultured hepatocytes sourced from 1 male Wistar rat and transfected (400 µM for 4 hrs) with either: 1) siRNA for CAR (100 nM) + Lipofectamine RNAiMAX (4 µL) + serum/antibiotic free medium (200 µL) 2) negative control siRNA (100nM) + Lipofectamine RNAiMAX (4 µL) + serum/antibiotic free medium (200 µL) 3) untreated group - not exposed to transfection mixture	Cultured hepatocytes transfected with siRNA (CAR) and siRNA (control) were exposed for 2 days to: Metofluthrin: 50 µM Phenobarbitone: 50 µM Untreated hepatocytes were not exposed to Metofluthrin or phenobarbitone.	Analysis and quantification of isolated mRNA (CAR, CYP 2B1 and GAPDH (used to normalize levels of CAR and CYP 2B1)) was conducted using quantitative real time PCR. *Level of CAR mRNA expression:* Hepatocytes transfected with siRNA for CAR showed a 34-37% reduction in CAR mRNA expression compared to negative controls. *Levels of CYP 2B1 mRNA expression:* <u>Metofluthrin</u> Control: CYP 2B1 mRNA was significantly induced in control hepatocytes. siRNA for CAR: CYP 2B1 mRNA was induced in hepatocytes transfected with siRNA for CAR. However, this was only by 32% of that in the control. <u>Phenobarbitone</u> Control: CYP 2B1 mRNA was significantly induced in control hepatocytes. siRNA for CAR: CYP 2B1 mRNA was induced in Hepatocytes transfected with siRNA for CAR. However, this was only by	Methodology employed the RNA interference technique (RNAi) to reduce CAR mRNA levels in rat hepatocytes and thus examine the importance of CAR in the CYP2B induction stimulated by Metofluthrin and phenobarbitone. A dose of 50 µM Metofluthrin was selected because a Metofluthrin concentration of in the liver at 900-1800 ppm is estimated to be about 1-10 µM. A dose of 50 µM phenobarbital was selected as this concentration has been previously shown to induce CYP 2B-dependent enzyme activity in

		21% of that in the control.	cultured rat hepatocytes (Madan *et al*, 1999).
Hirose *et al* (2009). This study is divided in to two parts: 1) CYP INDUCTION STUDIES Primary hepatocyte cultures were obtained from male Wistar rats and a human female (39 years old) donor. 2) REPLICATIVE DNA SYNTHESIS STUDIES Primary hepatocyte cultures were obtained from male Wistar rats and two human female (aged 41 and 63 years) donors.	1) CYP INDUCTION STUDIES Rat and human hepatocytes were exposed (72 hrs) to either: Metofluthrin: 50 µM Phenobarbitone: 50 µM Control 2) REPLICATIVE DNA SYNTHESIS STUDIES Rat hepatocytes were exposed (48 hrs) to serum free medium containing either: Metofluthrin:10 -1000 µM Phenobarbitone:	1) CYP INDUCTION STUDIES cDNA was prepared from total RNA by reverse transcription polymerase chain reaction. Analysis and quantification of isolated mRNA (CYP 2B1, CYP 2B6 and GAPDH (used to normalize levels of CYP 2B1 and 2B6)) was conducted using quantitative real time PCR. **Measurement of CYP 2B1 in rat hepatocytes** Metofluthrin: 3.4 fold increase in CYP 2B1 levels compared with control Phenobarbitone: 3.7 fold increase in CYP 2B1 levels compared with control **Measurement of CYP 2B6 in human hepatocytes** Metofluthrin: 2.4 fold increase in CYP 2B6 compared with control Phenobarbitone: 16. 0 fold increase in CYP 2B6 compared with control **Measurement of the CYP 2B form of enzymatic markers 7-pentoxyresorufin O-depentylase** Phenobarbitone: significant increase in 7-pentoxyresorufin O-depentylase activity was observed in rat and human hepatocytes compared to control. Metofluthrin: a significant increase in 7-pentoxyresorufin O-depentylase activity was observed in human hepatocytes compared to control. An increase was also observed in human hepatocytes, however, this was not statistically significant. 2) REPLICATIVE DNA SYNTHESIS STUDIES DNA synthesis was determined by measuring the amount of BrdU incorporation into DNA over the last 24 hours of the treatment period for rat and human hepatocytes. **Measurement of DNA synthesis in the rat hepatocytes** Phenobarbital: significant increase in replicative DNA synthesis at 100-500 µM. Metofluthrin: significant increase in replicative DNA synthesis at 10-1000 µM **Measurement of DNA synthesis in human**	Metofluthrin concentration (50 uM) was selected based on the results of *in vivo* studies where liver levels of were determined (Deguchi *et al*, 2009) and *in vitro* studies where no cytotoxicity was observed in cultured hepatocytes. Both human and rat hepatocytes responded appropriately when stimulated with a growth factor mitogen.

	10-1000 µM Control Human hepatocytes were exposed (48 hrs) to serum free medium containing either: Epidermal growth factor (EGF): 1-50 ng/ml Hepatocyte growth factor (HGF): 1-100 ng/ml Metofluthrin:10 -1000 µM Phenobarbitone: 10-1000 µM 5 ng/ml of EGF was added to the media of human hepatocytes treated with HGF, Phenobarbital and Metofluthrin. 10 µM 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.	**hepatocytes** Metofluthrin: no effect on replicative DNA synthesis Phenobarbital: no effect on replicative DNA synthesis Epidermal growth factor: significant concentration dependent increase in replicative DNA synthesis at 5-50 ng/ml. Hepatocyte growth factor: significant concentration dependent increase in replicative DNA synthesis	
Yamada 2012 (unpublished). This study is divided into two parts: 1) CYP INDUCTION STUDIES Primary human hepatocyte cultures were obtained from 2 male (aged 26 and 66 years) and 2 female (aged 52 and 38) donors	1) CYP INDUCTION STUDIES Human hepatocytes were exposed (48 hrs) to either: Metofluthrin: 5, 10, 50, 100, 500 or 1000 µM Phenobarbitone:	1) CYP INDUCTION STUDIES cDNA was prepared from total RNA by reverse transcription polymerase chain reaction. Analysis and quantification of isolated mRNA (CYP 2B6 and GAPDH (used to normalize levels of CYP 2B6)) was conducted using quantitative real time PCR. **Measurement of CYP 2B6** Metofluthrin: 1.9-fold increase in CYP 2B6 levels at 1000 µM compared with control. Phenobarbitone: 5-fold increase in CYP 2B6	Cytotoxicity (assessed via the MTT assay) was not observed at any dose level.

	1000 µM 0.5 ng/ml of EGF was added to the media of human	levels compared with control.	
2) REPLICATIVE DNA SYNTHESIS STUDIES Primary human hepatocyte cultures were obtained from 2 male (aged 26 and 66 years) and 2 female (aged 52 and 38) donors	hepatocytes treated with Phenobarbital and Metofluthrin. 2) REPLICATIVE DNA SYNTHESIS STUDIES Human hepatocytes were exposed (48 hrs) to either: Metofluthrin: 10, 50, 100, 500 or 1000 µM Sodium Phenobarbitone: 100, 500, 750 or 1000 µM Hepatocyte growth factor: 1, 10 or 100 ng/ml 0.5 ng/ml of EGF was added to the media of human hepatocytes treated with HGF, Phenobarbital and Metofluthrin. 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.	<u>2) REPLICATIVE DNA SYNTHESIS STUDIES</u> DNA synthesis was determined by measuring the amount of BrdU incorporation into DNA over the last 24 hours of the treatment period for human hepatocytes. **Measurement of DNA synthesis in human hepatocytes** <u>Metofulthrin</u>: no effect on average replicative DNA synthesis up to 1000 µM <u>Phenobarbital</u>: no effect on average replicative DNA synthesis <u>Hepatocyte growth factor</u>: significant concentration dependent increase in average replicative DNA synthesis	

In the first study, fresh hepatocytes were isolated from a single untreated male Wistar rat and incubated in serum-free conditions with CAR siRNA (CAR small interfering RNA, 100nM) for 4 hours (Deguchi *et al* 2009). The medium was then changed to a standard hepatocyte serum-based medium supplemented with Metofluthrin or phenobarbitone (50 µM). Two controls were

established, completely untreated hepatocytes and hepatocytes treated with siRNA. After a further 2-day incubation period, total RNA was isolated, reverse transcribed to cDNA and assayed for CAR and CYP 2B1 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 (34-37% of untreated controls) was observed in hepatocytes treated with CAR siRNA. When hepatocytes pre-treated with CAR siRNA were induced with Metofluthrin or phenobarbitone, statistically significant decreases in CYP2B1 mRNA were observed (21% and 32% of silenced control, with phenobarbitone and Metofluthrin respectively). Overall, this study indicates that the CAR is involved in the induction of CYP 2B1 mRNA.

In a second *in vitro* study, the effects of Metofluthrin and phenobarbitone on CYP 2B (CYP 2B1 - rat or CYP 2B6 - human) mRNA expression and enzyme specific activity, and DNA replication, in human and rat hepatocytes (Hirose Y *et al* 2009) were investigated.

Primary cultures of human and rat hepatocytes were incubated for 18 hours with Metofluthrin or phenobarbitone (50 µM). At the end of this period, the cells were assayed for CYP 2B1 or 2B6 mRNA using real time quantitative PCR. Additional investigations for the relevant CYP 2B specific activity were also conducted.

Compared to controls, CYP 2B1 mRNA was increased in rat hepatocytes (3.4 and 3.7-fold with Metofluthrin and phenobarbitone respectively) and CYP 2B6 mRNA increased in human hepatocytes (2.4 and 16.0-fold with Metofluthrin and phenobarbitone respectively). CYP 2B1 specific activity was increased in rat (25 and 37% with Metofluthrin and phenobarbitone respectively) and CYP 2B6 specific activity increased in human hepatocytes (25 and 80% with Metofluthrin and phenobarbitone respectively).

In the same study as described above the effect of Metofluthrin or phenobarbitone on hepatocyte replicative DNA synthesis was investigated by BrdU labeling. Primary rat hepatocytes were incubated for 48-hours in serum free medium containing 10ng/ml epidermal growth factor (EGF) and either phenobarbitone or Metofluthrin. Primary human hepatocytes from 2 female donors were incubated for 48-hours in serum free medium containing hepatocyte growth factor, phenobarbitone or Metofluthrin, plus 5 ng/ml EGF.

Replicative DNA synthesis was statistically significantly increased in rat hepatocytes treated with Metofluthrin at concentrations of 10 µM and above (by around 50% compared to controls), and in phenobarbitone treated rat hepatocytes at 100 and 500 µM (by around 20%). Human hepatocytes failed to respond when tested with either Metofluthrin or phenobarbitone. However, the human hepatocytes gave a clear positive dose-response when incubated with hepatocyte growth factor (0-100 ng/ml) in the presence of 5 ng/ml EGF, indicating that they had the potential to respond.

In another study, conducted to provide additional information on the potential of Metofluthrin to induce CYP 2B6 mRNA levels and replicative DNA synthesis, primary human hepatocytes were isolated from an additional 4 donors and tested following a similar protocol as for Hirose et al 2009. In this study, CYP 2B6 investigations were conducted using Metofluthrin (0-1000 µM) and phenobarbitone (1000 µM), and the replicative DNA synthesis investigation using Metofluthrin and phenobarbitone (0-1000 µM).

Compared to controls, mean increases in CYP 2B6 mRNA levels were observed with Metofluthrin, (1.5 to just 1.9-fold), at concentrations of 50 µM and above, but 1000 µM phenobarbitone induced a 5-fold increase. Only the increase observed with phenobarbitone achieved statistical significance.

Human hepatocytes failed to mount a proliferative response (replicative DNA synthesis) when tested with either Metofluthrin or phenobarbitone. In contrast, a clear dose-response was observed when human hepatocytes were incubated with hepatocyte growth factor (0-100 ng/ml) in the presence of 5 ng/ml EGF, indicating that they had innate proliferative capacity.

***In vivo* studies**

Test system	Dose levels	Results
Deguchi et al (2009). Oral (dietary) Rat, Wistar (Male and Female) 5-7/sex/group 7 day study Alzet minipumps containing BrdU with a release rate of 200 mg/hr were implanted into the subcutaneous tissue of rats under anaesthesia on the day prior to the 7 days of the scheduled sacrifice. Main measurement; gene expression profiling, liver weight, liver histology, hepatocellular DNA synthesis (proliferation) and CYP expression and activities	Treatment group: exposed to 0, 200, 900, 1800 or 3600 ppm Metofluthrin for 7 days days estimated to be 0, 13, 64, 121 or 221 mg/kg/day in males, and 0, 13, 58, 96 or 149 mg/kg/day in females Positive control: 1000 ppm sodium phenobarbitone (NaPB) estimated to be 64 mg/kg/day in males and 67 mg/kg/day in females Recovery group: exposed to 0, 200, 900, 1800 or 3600 ppm Metofluthrin or 1000 ppm NaPB for 7 days and untreated for 7 days Positive control: 1000 ppm sodium phenobarbitone (NaPB)	**Gene expression profiling** Gene expression profiling was determined for the livers of three male rats from each of the control, 1800 ppm Metofluthrin and 1000 ppm NaPB treatment groups. Microarray data analysis (performed using the Affymetrix protocol and Affymetrix data suit) revealed that exposure to Metofluthrin and NaPB caused the upregulation of 25 and 85 genes and downregulation of 10 and 14 genes, respectively. Clustering analysis was used to investigate the genes altered by Metofluthrin treatment and demonstrated that the profile of the altered genes was generally similar between Metofluthrin and NaPB treated animals. Most of the genes that were upregulated by Metofluthrin were metabolic enzymes including glutathione-S-transferase, CYPs and UDP-glucosyltransferase (UGTs). These genes were also upregulated by NaPB with greater potency. **CYP expression** cDNA was prepared from total RNA by reverse transcription polymerase chain reaction. Analysis and quantification of isolated mRNA (CYP 2B1/2, CYP 3A1 and CYP 3A2) was conducted using quantitative real time PCR. **Clinical signs and histopathology** 3600 ppm- tremor, two females of the treatment group and one male of the recovery group were found dead suggesting that this dose exceeded the MTD. Increased liver weights associated with enlarged/dark liver and centrolobular hepatocellular hypertrophy, but no cytotoxicity. Electron microscopy revealed dilation and/or proliferation of SER but not proliferation of peroxisomes. 1800 ppm- Increased liver weights associated with enlarged/dark liver and centrolobular hepatocellular hypertrophy, but no cytotoxicity. Electron microscopy

		revealed dilation and/or proliferation of SER but not proliferation of peroxisomes. **Hepatic activity of 7-pentoxyresorufin O-dependentylase (CYP 2B marker) and testosterone 6β-hydroxylase (CYP 3A marker)** The microsomes from control, 3600 ppm Metofluthrin and 1000 ppm NaPB treatment groups were used for measurement of enzyme activities. Hepatic microsomal 7-pentoxyresorufin O-dependentylase activity was determined by fluorometric analysis which revealed that: Metofluthrin: increased activity in both male and female rats compared to control. NaPB: increased activity in both male and female rats compared to control Hepatic microsomal testosterone 6β-hydroxylase was determined by high performance liquid chromatography (HPLC) analysis which revealed that: Metofluthrin: increased activity in female rats compared to control. NaPB: increased activity in both male and female rats compared to control. **Hepatocellular DNA synthesis (proliferation)** Hepatocyte cell replicative DNA synthesis was determined as BrdU- labeling incidences Metofluthrin: statistically significant increase in BrdU-labelling incidences in males at 900 and 1800 ppm NaPB: increase in BrdU-labelling incidences in both sexes at 1000 ppm NaPB. All of these changes were reversible on cessation of treatment
Deguchi et al. (2009) Oral (dietary) Rat, Wistar, Males; 5/group 7 and 14 days Main measurement; liver weight, liver histology, hepatocellular DNA synthesis (proliferation) and CYP expression and	0 and 2700 ppm Metofluthrin, estimated to be 134 mg/kg/day Sodium phenobarbitone (positive control) at an estimated dose of 62 mg/kg/day	Metofluthrin: increased relative liver weight, CYP P450 2B1 mRNA after 1 and 2-weeks, and elevated BrdU labeling index after week 1 only. No changes reported with phenobarbitone.

activities		
Deguchi Y (2009) Oral (dietary) Rat, Wistar, M, F 8/group 7 days, Apoptosis oxidative stress and gap junctional intracellular communication	0, 200, 900, 1800, 3600 ppm Estimated to be 0, 14, 62, 127 or 254 mg/kg/day in males, and 0, 15, 65, 144 or 263 mg/kg/day in females Sodium phenobarbitone (positive control) at an estimated dose of 68 mg/kg/day in m ales and 78 mg/kg/day in females	Metofluthrin: Significant decrease in gap-junctional intracellular communication No clear adverse effects on lipid peroxidation, a marker of oxidative stress Increases in total glutathione (GSH) and reduced GSH (GSSG) at doses ≥ 54 – 68 mg/kg Phenobarbitone: Decrease in gap-junctional intracellular communication Decease in the extent of apoptosis was noted in males only

In the first study groups of rats (Wistar strain, 5-7/dose) were administered Metofluthrin in the diet at doses of 0, 13, 58-64, 96-121 and 149-221 mg/kg/day for one week prior to scheduled sacrifice (Deguchi *et al* 2009). Additional groups of treated animals were included to assess the effects of a one-week recovery period on control diet. A group of positive control animals was also included, administered phenobarbitone at an estimated dose of 64-67 mg/kg/day. A limited number of general toxicity investigations were conducted in addition to a cell proliferation assay (BrdU labeling index), determination of CYP 2B1 mRNA and protein levels, and CYP 2B1 enzyme assay.

Three mortalities occurred at the highest dose of 149-221 mg/kg/day in Metofluthrin treated animals, 2 females during treatment and one male during the recovery period. No treatment-related changes in body weight and food consumption were observed. Minor increases in absolute liver weights were observed in Metofluthrin treated animals at doses of 96-121 mg/kg/day and above, in males (by 6-13% compared to controls) and females (by 7-19%). In phenobarbitone treated animals, absolute liver weights were statistically significantly increased by around 20% in both males and females, compared to controls. Histopathological examination found a statistically significant increase in the incidence of hepatocyte hypertrophy in males dosed with phenobarbitone (4/5 compared to 0/5 in controls) only. No information on hyperplasia was provided.

The liver gene expression profile analysis found that the most prominent upregulated genes, in animals dosed with Metofluthrin and phenobarbitone were those directly or indirectly involved in Phase 1 and Phase II xenobiotic metabolism.

Compared to controls a statistically significant increase in relative mRNA levels of CYP2B1/2 was observed in males administered 121 mg/kg/day and above (6 and 10-fold at121 and 221 mg/kg/day, respectively) and in females administered 58 mg/kg/day and above (5, 13 and 18 fold at, 58, 96 and 149 mg/kg/day, respectively). Phenobarbitone induced 64 and 125-fold increases in CYP2B1/2 mRNA in females and males respectively. When compared to controls, a statistically significant increase in the level of CYP 2B protein was observed in males and females administered Metofluthrin at the top dose only (2-2.4-fold at 149-221 mg/kg/day). Phenobarbitone induced 8 and 23-fold increases in CYP2B protein in females and males respectively. An enzyme assay for functional 2B protein found statistically significant increases in CYP 2B1 enzyme activity in high dose Metofluthrin treated males (2.5-fold); and in males and females dosed with phenobarbitone (32

and 73-fold in females and males respectively). No enzyme assay information was provided for animals receiving lower doses of Metofluthrin.

Compared to controls, BrdU labeling indices were statistically significantly increased in Metofluthrin treated males at doses of 64 mg/kg/day and above (around 20% compared to 10% in controls) and in males and females administered phenobarbitone (30-40% compared to10-20% in controls). It has been suggested (by the authors) that the lack of a response in females could be due to small number of animals tested and wide inter-individual differences in response.

In a second study, rats (male Wistar 5/group) were dosed with Metofluthrin or phenobarbitone at doses of 121 mg/kg/day or 360 mg/kg/day respectively for 1 and 2 weeks (Deguchi *et al* 2009). A single group untreated controls was also included. Investigations were limited to liver weight changes, hepatocellular proliferation and CYP 2B expression. No adverse effects on body weight or food consumption were reported nor were there any mortalities.

Compared to controls, statistically significant increases in relative liver weights were reported in Metofluthrin treated animals (by 10 and 22% after 1 and 2 weeks respectively). Relative to controls, CYP 2B1 mRNA levels were statistically significantly increased after 1 and 2 weeks (by 12 and 5-fold respectively). Cell proliferation (by BrdU labeling index) was statistically significantly increased after 1 week (4% in controls compared to almost 9% in treated animals) but comparable to controls by week 2. No data on the phenobarbitone treated animals were reported.

In the final study to investigate other events associated with the CAR-mediated MOA, male and female rats were administered Metofluthrin or phenobarbitone for 1 week following the same regimen as the study reported above. At study termination, the effects of Metofluthrin and phenobarbitone treatment on gap-junctional intracellular communication, markers of oxidative stress and apoptosis were investigated. In addition some limited investigations on general toxicity were conducted.

Three females died at the highest dose of 247 mg/kg/day, during the recovery period. No treatment-related changes in body weight and food consumption were observed. Absolute liver weights were statistically significantly increased in Metofluthrin treated animals at doses of 94-127 mg/kg/day and above, in males (by 9-12% compared to controls) and females at the highest dose of 247 mg/kg/day (by 28%). In phenobarbitone treated animals, absolute liver weights were statistically significantly increased in both males (by 16%) and females (by 28%), compared to controls.

When compared to controls, gap-junctional intracellular communication, measured by the extent of fluorescent dye transfer was statistically significantly decreased in the livers of Metofluthrin and phenobarbitone-treated animals. In males, at doses of 127 mg/kg/day (80 µM compared to 120 µM in controls) and in females at a dose of 144 mg/kg/day and above (77 and 81 µM compared to 110 µM in controls). In phenobarbitone-treated animals gap-junctional intracellular communication was statistically significantly decreased in males and females (60 and 50 µM respectively, compared to 120 µM in controls). A clear decease in the extent of apoptosis was noted only in males dosed with phenobarbitone (0.63 compared to 1 in controls). Inhibition of apoptosis is considered a key event in the MOA of phenobarbital-induced liver tumours (Holsappe et al, 2006; Whysner et al, 1996) and so the lack of response could mean that the method employed lacked the sensitivity of morphological procedures.

There were no clear adverse effects of Metofluthrin on lipid peroxidation, a marker of oxidative stress. However, Metofluthrin caused statistically significant increases in total glutathione (GSH – by around 5-7 mM compared to 5 mM in controls) and reduced glutathione (GSSG – by around 6-7

mM compared to 4 mM in controls) at doses of 54-68 mg/kg/day and above. It is possible that the increase in GSSG could indicate that the hepatocytes are experiencing some oxidative stress.

Consideration of other potential modes of action

Liver tumours can be produced in rats by either genotoxic or non-genotoxic mechanisms. In the mutagenicity studies presented, Metofluthrin did not elicit a positive response in vitro or in vivo indicating a lack of genotoxocity. Non-genotoxic MOAs include cytotoxicity, activation of CAR, activation of PPARα, porphyria and hormonal perturbation. In the general toxicity studies there was no evidence of hepatoceullar toxicity (e.g. necrosis or fatty liver), peroxisome proliferation, porphyria or any evidence of hormonal perturbations. Gene expression profiling analysis studies carried out by Yamada et al (2006) showed that unlike effects on the CAR, there were no marked alterations in either PPARα, aryl hydrocarbon receptor (AhR) signaling (there was no induction of CYP1A1, 1A2, 1B1, 4A1, 4A2 or 4A3) or pregnane x receptor (PXR) signaling (no induction of CYP3A1 or 3A2).

Considering all the data together, the results suggest that Metofluthrin 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 perturbation or porphyria that could also explain its carcinogenicity in rats. The proposed explanation is that Metofluthrin 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. These data are consistent with findings from other pyrethroids, in particular momfluorothrin S-1563.

Data gaps, uncertainties and inconsistencies

The CAR-dependency for the stimulation of cell proliferation by Metofluthrin has not been established. This evaluation is not deemed essential as the critical role of CAR in the proposed MOA has been demonstrated previously by Wei *et al* 2000 and Yamamoto *et al* 2004. Therefore this data gap is not considered to alter the overall postulated MOA for Metofluthrin-induced liver tumours in the rat. There is no data on the role of effects on apoptosis in the MOA for Metofluthrin-induced liver tumours in the rat. This again is not considered to represent a significant data gap.

Non-genotoxic chemicals which are activators of CAR may produce liver tumours in both rats and mice, with the mouse generally appearing to be more susceptible (Lake, 2009). Metofluthrin was not observed to cause tumours in the mouse study, however, there are other examples of non-genotoxic CAR activators that produce liver tumours in the rat but not the mouse e.g. natural pyrethrins. In a two week study in male and female CD-1 mice treated with Metofluthrin or phenobarbital small increases in liver weight, centrolobular hepatocyte hypertrophy and induction of CYB2B marker enzyme activity were noted in both sexes (Sommer, 2003, 2004) (refer to Annex I for study details). However, these effects were not as marked as they were in the rat. The lack of tumour formation by Metofluthrin in the mouse could be due to the fact that the increases in liver weight, hypertrophy and CYP2B enzymes were only small, even at relatively large doses. The apparent species difference between the rat and the mouse in the effect of liver tumour formation may be attributable to a species difference in the metabolism and/or disposition of this compound.

Relevance to humans

Key (K) and Associative (A) Event	Evidence in Rats	Evidence in Humans
Activation of CAR (K)	Inferred from CAR-siRNA studies and from induction of CYP2B enzymes	Probable at high doses based on the induction of CYP2B in vitro
Induction of CYP2B (A)	Direct experimental evidence in vivo and in vitro in cultured hepatocytes	Probable at high does (Observed in cultured hepatocytes)
Hypertrophy (A)	Direct experimental evidence in vivo	None in this study but possible at high doses based on studies in humans given anticonvulsant drugs
Increased hepatocellular proliferation (K)	Direct experimental evidence in vivo and in vitro in cultured hepatocytes	Not predicted (not observed in vitro in cultured hepatocytes)
Altered hepatic foci (K)	Direct experimental evidence in vivo	Not predicted (no evidence)
Liver tumours (K)	Observed in vivo	Not predicted (no evidence)
Inhibition of gap junctional intercellular communication (A)	A decrease observed in vivo	No evidence in this submission
Decreased Apoptosis (A)	No evidence in this submission	No evidence in this submission

Overall, the differences between rat and human data with Metofluthrin were consistent with other compounds with a CAR-mediated MOA (Elcombe et al 2014).

Statement of confidence

After consideration of all available data, the Metofluthrin-induced activation of CAR is presented as a plausible explanation for the formation of liver tumours in rats. There is strong evidence that Metofluthrin causes activation of the CAR resulting in increased cell proliferation and the development of altered hepatic foci in rats. The induction of CYP2B enzymes and liver hypertrophy comprise associative events and represent reliable markers of CAR activation. Human data suggests that whilst Metofluthrin is capable of inducing CYP2B6 and causing hypertrophy in human hepatocytes, it does not lead to an increase in cell replication, a prerequisite for tumour formation. Therefore, the data strongly support the conclusion that qualitatively the CAR-mediated mode of action would not occur in humans following exposure to Metofluthrin.

4.10.4 Summary and discussion

Lifetime dietary administration of Metofluthrin induced a clear increase in liver tumours (adenomas and carcinomas) in male and female rats. The incidence of liver carcinomas in males exceeded both the concurrent and historical control incidence at doses of 38 mg/kg/day and above. In both sexes, administered the highest dose of 78-96 mg/kg/day terminal body weights were decreased compared

to controls but are not considered to represent a toxicologically significant exceedence of the MTD. It is also noted that the observed tumours were late onset and occurred at a single site. No treatment-related increases in tumour incidence were observed following lifetime dietary administration in mice.

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

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

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

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

The mechanistic studies (described in section 4.10.3) indicated that Metofluthrin is a CAR activator, inducing CYP 2B1/2 and hepatocellular proliferation, *in vivo*, in rats.

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

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

4.10.5 Comparison with criteria

In accordance with the CLP criteria, Cat 1A is not appropriate, as there is no evidence of carcinogenicity in humans. Classification in Carc 1 B is not justified as the animal data are limited rather than sufficient due to tumour incidence being restricted to one species (rats), in a single tissue with no evidence of a genotoxic mode of action. Taking into account the in vitro and in vivo mechanistic data discussed above, Carc. 2 is not justified, as the data indicate that the liver tumours caused by Metofluthrin in rats are induced by CAR activation, a mechanism which is not relevant for human health. Therefore, in this instance no classification appears to be most appropriate.

4.10.6 Conclusions on classification and labelling

Not Classified – Conclusive but not sufficient for classification.

4.11 Toxicity for reproduction

4.11.1 Effects on fertility

The potential for Metofluthrin to adversely effect fertility has been investigated in rats in a standard dietary multigeneration study and non-standard gavage studies.

4.11.1.1 Non-human information

Table 23: Summary table of relevant reproductive toxicity studies

Method	Results/Remarks	Reference
OECD TG 416 Diet Sprague Dawley rats, 30/sex/dose 10 weeks pre-mating then through 2 successive generations 0, 50, 200, 1000 & 1800 ppm In F0 animals estimated to be: 0, 3.5, 15, 69 & 126 mg/kg/day in males, 0, 3.9, 16, 77 & 140 in females during premating period ,0, 7.6, 29, 146 & 243 mg/kg/day in females on lactation days 0 – 14. In F1 animals estimated to be: 0, 4.4, 18, 89 & 166 mg/kg/day in males, 0, 4.9, 20, 98 & 184 in females during premating period, 0, 8.4, 31, 163 & 283 mg/kg/day in females on lactation days 0 – 14. GLP	No treatment-related effects on fertility. Clinical signs of toxicity were confined to a statistically significantly increased incidence of tremor and twitching observed in lactating females at the highest dose of 1800 ppm in F0 animals (tremor 23/30 animals; twitch 22/30 animals); and at 1000 and 1800 ppm in F1 animals (tremor 14/30 and 30/30 animals, respectively; twitch 13/30 and 30/30 animals, respectively). At 1000 and 1800 ppm, a statistically significant increase in liver weight was reported in males of the F0 generation (absolute, 16 % and 18 %, respectively; relative, 16 % and 22 %, respectively) and the F1 generation (absolute, 18 % and 12 %, respectively). In females, relative liver weight was statistically significantly increased at 1800 ppm in the F0 generation (6 %); while absolute liver weight was increased at 1000 and 1800 ppm in the F1 generation (14 % and 18 %, respectively). These liver weight changes were also associated with increased incidences of hepatocellular hypertrophy. There were no clearly treatment-related changes on body weight or food consumption There were no adverse effects on any fertility parameters investigated in this study. No treatment-related effects on sperm parameters, mating performance, fertility or parturition were observed in this study. In F1 animals of the 1800 ppm group, statistically significant retardations of preputial separation (45.1 days in controls compared to 49.2 days) and vaginal opening (34.5 days in controls compared to 36.3 days) were observed. However, when these changes were reanalysed for statistical significance with pup weight as a co-variant, no treatment-related changes were apparent, suggesting that these retardations are secondary to pup weight decreases and not treatment-related. Statistically significantly decreases in pup weights were reported in the 1800 ppm group from around day 21 post-partum in F1 pups (equivalent dose level 140 mg/kg/d) and from day 10 post-partum in F2 pups (equivalent dose level 184 mg/kg d). By day 28, both F1 and F2 pup weights were decreased by approximately 15 % when compared to control animals.	A6.8.2(03) Doc IIIA Hoberman, A. (2005)

Non standard Gavage dosing Sprague Dawley rats, 20/sex/dose 2 weeks pre-mating until pregnancy day 7 (females), or until female sacrifice (males) Males: 0, 5, 10 or 20 mg/kg/day Females 0, 10, 20 or 40 mg/kg/day GLP Non-guideline, but considered to be acceptable during the biocides review	Maternal tremor and death at top dose level. No other treatment-related effects, including on fertility parameters.	A6.8.2(01) Doc IIIA Hara, H. (2002b)

4.11.1.2 Human information

There is no human information available.

4.11.2 Developmental toxicity

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

4.11.2.1 Non-human information

Table 24: Summary table of relevant developmental toxicity studies

Method	Results/Remarks	Reference
EC B31 Gavage Rat, Sprague Dawley, 24 dose Days 6-19 of gestation 0, 5, 15, 30 mg/kg/day in corn oil GLP	Maternal: post-dosing tremor at the top dose Developmental toxicity: No skeletal or soft tissue malformations or variations	A6.8.1(01) Doc IIIA Hara, H. (2002a)
EC B31 Gavage Rabbit, New Zealand White, 24 dose Days 6-27 of gestation 0, 25, 125, 250 mg/kg/day in corn oil GLP	Maternal: Mortality at 125 mg/kg/day and above Developmental toxicity: No skeletal or soft tissue malformations or variations	A6.8.1(02) Doc IIIA Horie, N. (2002)
Non Standard Gavage Rat, Sprague Dawley Day 6 of gestation to day 20 post partum 0, 5, 15, 30 mg/kg/day (in corn oil) GLP Non-guideline, but considered to be acceptable during the biocides review	Maternal tremor and death at top dose level. No other treatment-related effects	A6.8.2(02) Doc IIIA Hara, H. (2002c)

4.11.2.2 Human information

There is no information available.

4.11.3 Other relevant information

4.11.4 Summary and discussion of reproductive toxicity

Fertility

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

Developmental toxicity

No evidence of developmental toxicity was observed in rats, at doses of up to 30 mg/kg/day, the highest dose tested. At this dose, maternal toxicity was manifested by post-dose tremor (5/24 animals). Similarly, no evidence of developmental toxicity was observed in rabbits at doses of up to 250 mg/kg/day, the highest dose tested. In rabbits, Metofluthrin caused a single maternal death at 125 and 250 mg/kg/day.

In another study, pregnant females were exposed from gestation day 6 to day 20 post-partum. This study also included an assessment of the behavioral and reproductive development of the pups. Post-dosing tremor was reported in 15/24 top dose animals (30 mg/kg/day); similar effects were not observed at 15 mg/kg/day and below. No effects were reported on pregnancy, parturition, ability to rear young or on the development and reproductive performance of the offspring.

4.11.5 Comparison with criteria

As there are no data in humans, and no evidence of developmental toxicity or an adverse effect on reproductive performance in relevant animal studies, Metofluthrin does not meet the criteria for classification.

4.11.6 Conclusions on classification and labelling

Not Classified – conclusive but not sufficient for classification

4.12 Other effects

No additional information.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Metofluthrin is a synthetic pyrethroid insecticide intended for killing adult mosquitoes. Available environmental fate and hazard studies for Metofluthrin have been reviewed under Directive 98/8/EC. The studies are summarised in the Competent Authority Report (CAR) dated June 2010.

For the CAR, the substance was originally considered to be a mixture of 8 isomers and the purities quoted in that document related to the sum of all 8. Following discussions under Directive 98/8/EC it was agreed that it should be considered to have a single main component (the 'RTZ' isomer or Epsilon-metofluthrin, present at > 75.4 % w/w). The relative 'RTZ' purity for the batches tested is shown below:

PK-020301G	82.2 %	Ecotoxicology Studies
PK-010501G	80.2 %	Ecotoxicology Studies
KA-0622	Not analysed	Biodegradation and BCF Studies

Environmental effects of Metofluthrin were established using both radiolabelled and non-radiolabelled forms of Metofluthrin as either the technical grade mixture of all isomers (S-1264) containing >80% RTZ isomer, or the pure RTZ or RTE isomers of metofluthrin. Table 25 sets out how the various test substances are referred to in the following text. In addition throughout this section RTZ and RTE isomers may simply be referred to as Z and E isomers.

Table 25: Metofluthrin abbreviations

Species	Abbreviation
metofluthrin (**all isomers**)	S-1264
metofluthrin RTZ isomer	S-1264-*Z*
metofluthrin RTE isomer	S-1264-*E*
Radiolabelled [Methoxymethylbenzyl-α-^{14}C]S-1264RTZ	Alc-*Z*
Radiolabelled [Carbonyl-^{14}C]S-1264RTZ	Acid-*Z*
Radiolabelled [Methoxymethylbenzyl-α-^{14}C]S-1264RTE	Alc-*E*
Radiolabelled [Carbonyl-^{14}C]S-1264RTE	Acid-*E*

5.1 Degradation

Table 26: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Aquatic hydrolysis OECD 111 GLP	Hydrolysis t1/2: 46 to 94.8 days at 12°C at pH 9 Stable at pH 4 and 7	Valid study	A7.1.1.1.1 Doc IIIA Ponte (2004a)
Aquatic photodegradation 91/414/EEC, Annex II, 2.9.2 and 2.9.3 as amended 95/36/EEC / SETAC GLP	Photolysis t1/2: 2.2 to 2.6 days between 40 and 50°N at pH 4	Valid study	A7.1.1.1.2 doc IIIA Ponte (2004b)
Ready Biodegredation OECD 301C GLP	2% mineralisation after 28 days	Valid study Not readily biodegradable	A7.1.1.1.2.1 Doc IIIA Matsumoto (2000)

5.1.1 Stability

Hydrolysis

A hydrolysis study in accordance with GLP and OECD Guideline 111 was performed using metofluthrin with a radiochemical purity of > 98 % (Ponte, 2004a). Initial tests had shown that there was no significant difference in the behaviour of the Z and E isomers and so just Alc-*Z* and Acid-*Z* were used for testing.

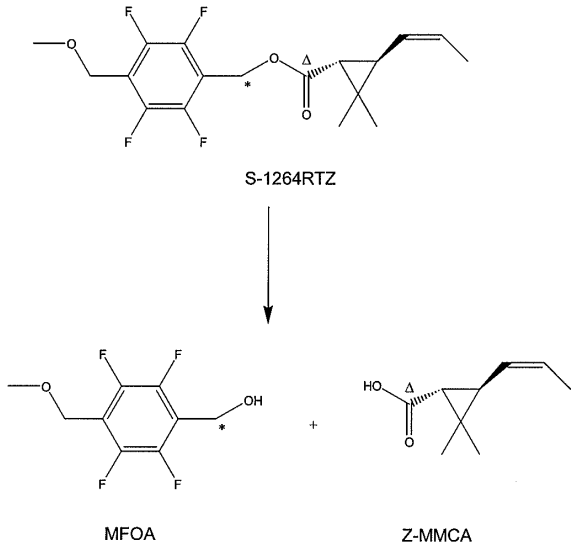
The preliminary test showed that Alc-*Z* was stable in pH 4 and pH 7 buffer systems and so no further testing at these pHs was considered necessary. Degradation did occur at pH 9, and a definitive test was performed at this pH. This used a test concentration of 0.2 mg/l and temperatures of 25°C for 33 days and 40°C for 8 days, respectively, using both the methoxymethylbenzyl-α-^{14}C (Alc-*Z*) and carbonyl-^{14}C labels (Acid-*Z*).

The half-lives of Alc-*Z* and Acid-*Z* determined at 25°C at pH 9 were reported as 30.7 and 33.5 days, respectively. At an elevated temperature (40°C) these half-lives were reduced to 4.9 and 5.1 days, respectively. These were extrapolated by calculation to provide the following half-lives at 12 °C:

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

Hydrolysis products were the corresponding carboxylic acid and alcohol formed through the cleavage of the ester, which are shown in figure 1 below.

Figure 1: Abiotic degradation results



Aqueous photolysis

A phototransformation in water test (Ponte, 2004b) was carried out in accordance with Council Directive 91/414/EEC, Annex II, 2.9.2 and 2.9.3 as amended by Commission Directive 95/36/EEC and as by The Society of Environmental Toxicology and Chemistry (SETAC). The test substances were Acid-*Z* and Acid-*E* with a purity > 95 %.

Test solutions of 0.2 mg/l Acid-*Z* and Acid-*E* isomers were kept at pH 4 and 20°C with exposure to either continuous light or dark conditions for 7 days. The irradiated samples were exposed to an average light intensity of 460 Wm^{-2} within the range of 300-800 nm using a Xenon arc lamp, corresponding to outdoor exposure. Samples were taken and analysed after 16 hours, and 1, 2, 4, 5 and 7 days' exposure.

The same test was conducted on the Alc-*Z* isomer although quantum yield was not determined for this species and there was no significant difference found in photolysis rate constant (k^c_p).

Acid-*Z* and Acid-*E* were shown to degrade rapidly when exposed to artificial light in sterile pH 4 buffer solution, declining to < 23 % of the applied dose at the end of the irradiation period

The major degradation products were the same as those reported for hydrolysis. For Alc-*Z* these were MFOA (24.4 % of applied dose); formed by photo-induced cleavage of the ester linkage and diol-1264 (13.5 % of applied dose); formed by oxidation of the double bond in the prop-1-enyl moiety. For Acid-*Z* and Acid-*E* isomers the major degradants observed were $^{14}CO_2$ (35.4 and 28.6 % of applied dose respectively) and diol-1264 (up to 16.7 % mean of applied dose).

Table 27: Aqueous Photolysis

Guideline / Test method		Total recovery of test substance [% of applied a.s.]*	Photolysis rate constant (k^c_p)	Direct photolysis sunlight rate constant (k_{pE}) (d)	Half-life ($t_{1/2E}$) (d)	Reference
91/414/EEC, Annex II, 2.9.2 and 2.9.3 as amended 95/36/EEC / SETAC	Acid-*Z*	88.4 – 102.7	0.2038 - 0.2567	0.29 (40 °N lat) 0.27 (50 °N lat)	2.4 (40 °N lat) 2.6 (50 °N lat)	A7.1.1.1.2 Doc IIIA Ponte, 2004b
	Acid-*E*	91.5 – 101.2	0.2390	0.31 (40 °N lat) 0.28 (50 °N lat)	2.2 (40 °N lat) 2.5 (50 °N lat)	

*Initial molar TS concentration 0.2 mg l^{-1} 10 % acetonitrile

Acid-*Z* and Acid-*E* degraded rapidly within the aqueous compartment to form three potential major degradation products over a 7 day period: MFOA, diol-1264 and $^{14}CO_2$.

5.1.2 Biodegradation

No data are available.

5.1.2.1 Biodegradation estimation

5.1.2.2 Screening tests

A 28-d MITI 1 (OECD 301C) ready biodegradation test (Matsumoto, 2000) was carried out using 99.2% purity metofluthrin. Test bottles for control, abiotic control, reference (aniline) and test substance were prepared using a sewage sludge inoculum and were sampled for dissolved oxygen concentration on days 7, 14, 21 and 28. Both the test substance and aniline solutions contained 30 mg/l of inoculum.

The degradation of metofluthrin based on theoretical oxygen demand was found to be very low (only 2 %) after 28 days' exposure. This was further supported by the mean residual rates of metofluthrin calculated from direct determination (GC), which were 96 % and 95 % for the test substance and abiotic control respectively.

The test system was shown to be valid as the degradation of the reference material (aniline) calculated from oxygen consumption, was 63 % after 7 days' exposure. Whilst a toxicity control was not included, an Activated Sludge Respiration Inhibition Study (refer to Section 5.4.4) indicates Metofluthrin is not toxic to micro-organisms.

5.1.2.3 Simulation tests

5.1.2.3.1 Aquatic tests

No data available.

5.1.2.3.2 Soil tests

A non-GLP aerobic soil degradation test was performed using Alc-*Z*, Acid-*Z*, Alc-*E* and Acid-*E* isomers, in accordance with the U.S. EPA Guideline, Subdivision N, Environmental Fate: Chemistry Series 162-1 (Kodaka *et al*,.) .

Two sandy loam soils; California and Mississippi (see Table 28 for characteristics), were used in a series of 20 g soil samples each treated with test substance and mixed to give a uniform concentration of 1 mg/l (dry soil weight basis). Test soils were then incubated in darkness at 25 ± 2 ºC for up to 120 days in sealed jars, which were continuously purged with CO_2-free air and the moisture content was adjusted to original levels once a month with distilled water. The effluent air was passed through as series of traps designed to recover any organic volatiles or carbon dioxide.

Table 28: Test soil characteristics

Characteristic	California soil	Mississippi soil
Soil classification (USDA)	Sandy loam	Sandy loam
Sand [%]	77	49
Silt [%]	14	48
Clay [%]	9	3
Organic matter [%]	1.0	0.5
pH (1:1 H_2O)	7.3	7.1
Cation exchange capacity (MEQ/100 g dry soil)	7.7	6.7
Bulk density (g cm^{-3})	1.3	1.3
1/3 bar moisture (%)	11.0	9.0

Samples for analysis of Alc-*Z* and Alc-*E* isomers were taken after 0, 1, 3, 7/8, 14, 30 and 59 or 60 d post-treatment in both soil types tested. For the Mississippi soil an additional sample was taken for analysis 120 days post-treatment. By contrast, analysis of Acid-*Z* and Acid-*E* isomers was undertaken for samples only on days 0, 1, 3, 7/8 and 14 post-treatment. The media within the traps were analysed at every sampling interval and /or every two weeks.

Tables 29 to 32 present measured ^{14}C values for the *Z* and *E* isomers of metofluthrin in the two soils types.

Table 29: Measured Degradation of [Alc-^{14}C]S-1264 (Alc-*E* and Alc-*Z*) in Mississippi soil

[Alc-^{14}C]S-1264RTE	**% of the applied ^{14}C**							
	Days after application							
	0	**1**	**3**	**8**	**14**	**30**	**60**	**120**
Volatile ^{14}C	n/a	0.3	1.0	3.3	5.9	13.6	25.0	47.9
CO_2	n/a	0.3	1.0	3.2	5.7	13.4	24.9	47.8
Soil ^{14}C								
MeOH extract								
S-1264	102.7	84.8	58.9	25.0	5.3	1.7	0.6	1.0
MFOA-D	nd	12.7	32.4	56.4	63.2	46.4	10.9	nd
TFPA	nd	nd	0.7	2.5	4.6	7.3	12.1	16.5
Acid extract								
S-1264	n/a	1.2	0.5	0.5	nd	nd	nd	nd
MFOA-D	n/a	1.1	3.5	5.2	6.0	5.8	1.8	nd
TFPA	n/a	0.8	2.7	8.3	15.5	26.5	42.3	40.6
Overall total*	104.6	103.7	103.5	102.6	101.6	103.7	96.4	112.5
[Alc-^{14}C]S-1264RTZ	**% of the applied ^{14}C**							
	Days after application							
	0	**1**	**3**	**7**	**14**	**30**	**59**	**120**
Volatile ^{14}C	n/a	0.3	1.2	3.1	7.0	18.6	35.7	56.0
CO_2	n/a	0.3	1.2	3.1	7.0	18.6	35.7	56.0
Soil ^{14}C								
MeOH extract								
S-1264	103.5	79.6	50.4	15.9	1.8	1.2	2.1	1.1
MFOA-D	nd	16.3	40.4	65.0	65.8	40.5	7.5	nd
TFPA	nd	nd	1.1	3.3	5.8	11.3	14.3	12.0
Acid extract								
S-1264	n/a	1.7	0.8	nd	nd	nd	nd	0.3
MFOA-D	n/a	1.5	2.9	4.0	10.2	3.3	0.3	nd
TFPA	n/a	nd	3.6	7.5	9.6	24.0	28.1	25.8
Overall total*	104.6	102.7	102.8	100.2	102.4	101.5	91.2	100.0

*including others and bound residue, n/a: not analysed, nd: not detected

Table 30: Measured Degradation of [Alc-^{14}C]S-1264 (Alc-*E* and Alc-*Z*) in California soil

[Alc-^{14}C]S-1264RTE	**% of the applied ^{14}C**						
	Days after application						
	0	**1**	**3**	**8**	**14**	**30**	**60**
Volatile ^{14}C	n/a	0.5	1.8	8.9	17.6	49.1	87.4
CO_2	n/a	0.5	1.8	8.8	17.6	49.0	87.4
Soil ^{14}C							
MeOH extract							
S-1264	102.6	73.2	32.6	8.5	2.3	1.0	0.4
MFOA-D	nd	21.5	54.7	58.9	47.1	21.7	5.6
TFPA	nd	nd	1.9	5.2	8.0	6.2	1.4
Acid extract							
S-1264	n/a	1.2	0.8	nd	0.4	nd	nd
MFOA-D	n/a	1.7	4.3	6.3	5.6	3.2	1.7
TFPA	n/a	1.2	4.6	11.6	15.2	16.7	2.8
Overall total*	104.8	103.4	103.6	103.7	99.8	103.6	105.6
[Alc-^{14}C]S-1264RTZ	**% of the applied ^{14}C**						
	Days after application						
	0	**1**	**3**	**7**	**14**	**30**	**59**
Volatile ^{14}C	n/a	0.4	1.6	6.3	17.9	48.3	88.6
CO_2	n/a	0.4	1.6	6.3	17.9	48.3	88.5
Soil ^{14}C							
MeOH extract							
S-1264	103.3	72.0	33.3	8.4	1.7	1.0	1.0
MFOA-D	nd	22.7	52.0	66.2	52.9	24.6	4.9
TFPA	nd	0.4	2.2	4.3	6.6	6.9	0.4
Acid extract							
S-1264	n/a	1.8	1.0	nd	nd	nd	nd
MFOA-D	n/a	1.6	4.3	6.2	5.3	3.8	3.0
TFPA	n/a	1.4	4.7	10.6	12.4	14.9	1.1
Overall total*	105.0	104.2	102.2	104.4	100.0	104.7	105.4

*including others and bound residue, n/a: not analysed, nd: not detected

Table 31: Measured Degradation of [Acid-^{14}C]S-1264 (Acid-*E* and Acid-*Z*) in Mississippi soil

[Acid-^{14}C]S-1264RTE	**% of the applied ^{14}C**				
	Days after application				
	0	**1**	**3**	**8**	**14**
Volatile ^{14}C	n/a	2.6	12.3	36.1	59.2
CO_2	n/a	2.6	12.3	35.9	58.6
Soil ^{14}C					
MeOH extract					
S-1264	99.8	89.7	61.7	27.4	6.4
*(E)*M7	nd	2.6	4.1	5.1	0.5
Acid extract					
S-1264	n/a	3.1	1.7	0.5	nd
*(E)*M7	n/a	1.0	2.4	1.3	nd
RT24	n/a	1.5	6.1	9.6	10.0
Overall total*	101.4	104.1	101.8	100.4	100.9
[Acid-^{14}C]S-1264RTZ	**% of the applied ^{14}C**				
	Days after application				
	0	**1**	**3**	**7**	**14**
Volatile ^{14}C	n/a	2.5	11.8	32.8	65.1
CO_2	n/a	2.5	11.8	32.6	64.6
Soil ^{14}C					
MeOH extract					
S-1264	103.8	85.9	57.0	24.8	5.2
*(Z)*M7	nd	3.6	9.6	5.8	nd
Acid extract					
S-1264	n/a	2.4	2.2	1.1	0.5
*(Z)*M7	n/a	0.4	3.2	2.6	nd
RT24	n/a	2.0	5.2	9.4	6.8
Overall total*	105.2	101.9	101.9	99.1	101.4

*including others and bound residue, n/a: not analysed, nd: not detected

Table 32: Measured Degradation of [Acid-^{14}C]S-1264 (Acid-*E* and Acid-*Z*) in California soil

[Acid-^{14}C]S-1264R	**% of the applied ^{14}C**				
	Days after application				
	0	**1**	**3**	**8**	**14**
Volatile ^{14}C	n/a	5.4	25.7	68.9	82.0
CO_2	n/a	5.4	25.6	68.6	81.7
Soil ^{14}C					
MeOH extract					
S-1264	99.0	78.9	40.9	9.1	4.2
*(E)*M7	nd	4.8	13.0	2.0	nd
Acid extract					
S-1264	n/a	3.2	1.7	nd	nd
*(E)*M7	n/a	1.4	2.0	nd	nd
RT24	n/a	1.1	2.6	4.6	nd
Overall total*	101.4	102.4	99.6	99.4	104.1
[Acid-^{14}C]S-1264RTZ	**% of the applied ^{14}C**				
	Days after application				
	0	**1**	**3**	**7**	**14**
Volatile ^{14}C	n/a	5.3	26.1	66.6	81.1
CO_2	n/a	5.3	26.0	66.5	80.9
Soil ^{14}C					
MeOH extract					
S-1264	100.4	79.2	36.5	9.3	3.4
*(Z)*M7	nd	5.7	19.3	3.0	nd
Acid extract					
S-1264	n/a	2.1	2.9	1.8	nd
*(Z)*M7	n/a	nd	2.6	nd	nd
RT24	n/a	1.8	1.4	1.0	nd
Overall total*	103.2	100.2	100.2	98.4	101.7

*including others and bound residue, n/a: not analysed, nd: not detected

Z and E isomers of metofluthrin were found to degrade rapidly in soil with no significant differences in metabolic profiles. Non-acid extractable residues were below 15% of the initially applied ^{14}C. The DT_{50} values are presented in Table 33 along with any degradation products identified during the test.

Table 33: Aerobic degradation of Z and E isomers of metofluthrin in soil

Guideline / Test method	Soil type	% Organic carbon	Application rate of test substance	DT_{50} (d)	Degradation products	Reference
U.S. EPA Guidelines, Subdivision N Chemistry: Environmental Fate: Section Series 162-1.	California Sandy Loam	0.58	1 mg kg^{-1} dry weight equivalent of soil	2.3 – 2.9	**Alc-*Z* and Alc-*E*:** MFOA-D: max. 65.2 – 76.0 % at day 7-14. TFPA max. 21.8 – 23.2 % at day 14-30 **Acid-*Z* and Acid-*E*:** M7: maxi. 6.5 – 21.9 % at day 3.	A7.2.1 Doc IIIA Kodaka *et al.*, (2003)
	Mississippi Sandy Loam	0.29		2.4 – 3.5	**Alc-*Z* and Alc-*E*:** MFOA-D: max. 65.2 – 76.0 % at day 7-14. TFPA max. 42.4 – 57.1 % at day 59-120 **Acid-*Z* and Acid-*E*:** M7: max. 6.5 – 21.9 % at day 3.	

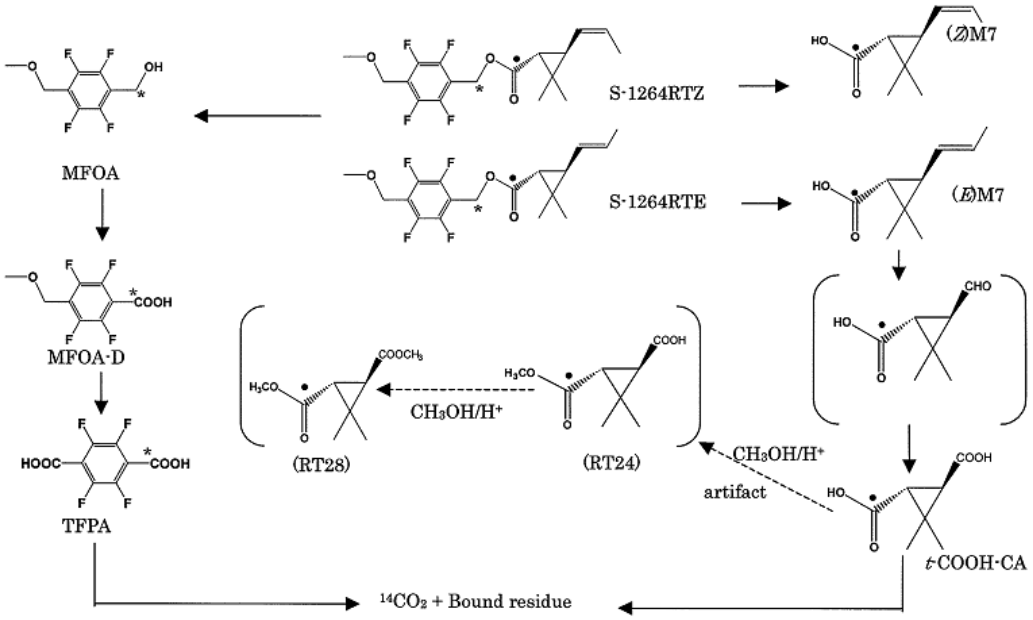
Z and E isomers of metofluthrin were shown to degrade via rapid cleavage of the ester linkage to form either MFOA or M7 as primary major metabolites. MFOA was immediately oxidised at the benzyl alcohol moiety to form a carboxyl group to give MFOA-D as a secondary major metabolite, which underwent further oxidation at the methyloxybenzyl moiety to form a tertiary major metabolite, TFPA. The other primary metabolite, M7, also under went oxidation at the prop-1-enyl moiety to form a carboxyl group. The degradation pathway is shown in Figure 2.

For the biocide CAR, the UK CA modelled the degradation of metofluthrin using measured data assuming non-linear degradation and single first order kinetics using Microsoft Excel software. This produced DT_{50} values for the primary degradation of metofluthrin of 1.93 – 2.52 days for California sandy loam soil and 2.76 – 4.19 days for Mississippi sandy loam soil. These values are comparable to the measured values of DT_{50} 2.3 – 2.9 days (California sandy loam) and 2.4 – 3.5 days (Mississippi sandy loam).

Overall *Z* and *E* isomers of metofluthrin were shown to primarily degrade in soil with half-lives of 2.3 – 3.5 days (at 25 ºC). The geometric mean of the Z isomer data is 2.6 days at 25 ˚C which equates to 7.4 days when converted to 12 ˚C using equation 25 of the TGD (EC, 2003).

Mineralization of *Z* and *E* isomers of metofluthrin to CO_2 occurred under the conditions tested at varying amounts (58.6-81.2% in 14 days for the acid-radiolabelled material, compared to 13.4-49% in 30 days for the alcohol-radiolabelled material). Intermediate major (> 10 %) metabolites MFOA-D and TFPA and M7 were identified with mean estimated half-lives of 52.1 days, 97 days and 1.7 days at 12 ºC respectively. The half-lives of the respective acid and alcohol primary metabolites are believed to explain the varied results observed in the test. As M7 (acid) has a very short half-life, mineralisation of the acid-radio-labelled metofluthrin occurs rapidly. MFOA (alcohol) has a longer half-life, and so mineralisation of the alcohol radio-labelled metofluthrin is slower.

Figure 2: Degradation kinetics as produced by Model Maker



Proposed Degradation Pathways of S-1264 in Soil under Aerobic Conditions.

5.1.3 Summary and discussion of degradation

In the environment Metofluthrin is generally hydrolytically stable, although slight hydrolysis will occur at pH 9. The substance is photodegradable, but this is not relevant for hazard classification.

Metofluthrin is not readily biodegradable following an OECD 301C study. A soil simulation study reported DT_{50} values of 2.3-3.5 days at 25°C equating to a mean DT_{50} of 7.4 days at 12°C. During the study the substance rapidly degraded to primary metabolites in soil. These mineralised to CO_2 with varying half-lives. The variation in mineralisation rates does not provide good evidence that Metofluthrin has an ultimate degradation half-life < 16 days (equating to 70 % degradation in 28 days).

Overall, Metofluthrin is considered not rapidly degradable for the purposes of hazard classification.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Organic carbon-water (K_{oc}) partition coefficient values have been calculated from two studies.

The first test (Walsh and Lentz, 2003c – A7.1.3(02) Doc IIIA)) analysed the adsorption of *E* and *Z* isomers using the standard HPLC estimation method (OECD 121 and GLP). The K_{oc} for Z and E isomers were determined as 16,000 and 17,000 l kg^{-1}, respectively (Table 34).

Table 34: HPLC established K_{oc} values

Compound	Retention Time			K'	Log k'	Calculated log K_{oc}*	K_{oc} l/kg
	Run 1	Run 2	Run 3				
S-1264-*E*	23.39	23.38	23.34	6.298	0.799	4.2	17,000
S-1264-*Z*	22.82	22.79	22.76	6.117	0.787	4.2	16,000

*estimated from linear regression analysis

The second method was a screening test, which was carried out using four soil types (loam, sandy clay loam, clay loam and sandy loam) in accordance with OECD 106 and GLP(Ponte see below). Alc-*Z* and Alc-*E* were used in the preliminary testing with a radiochemical purity of > 98 %. However, as no significant difference was noted between these isomers, the definitive test was carried out using Alc-*Z*. The results are shown in Table 35 below.

Table 35: Adsorption/desorption from soils

Guideline / Test method	Soil	Adsorbed a.s. [%]	l/kg					Degradation products		Reference
			K_a [1]	K_{aOC} [2]	K_d [3]	K_{dOC} [4]	K_a / K_d [5]	Name	[%] of a.s.	
OECD Guideline 106 U.S. EPA Guidelines 40	Loam	71.3 – 76.9	96.5	11,855	150	18,428	0.64	MFFO	0 – 4.4 % AR*	A7.1.3(01) Doc IIIA Ponte (2004c)
	Sandy clay	60.7 – 76.2	113	6,075	265	14,247	0.43			
	Clay loam	71.6 – 81.8	119	2,729	187	4,289	0.64	MFOA	0 – 5.2 % AR*	
	Sandy loam	69.4 – 69.9	54.6	4,075	67.3	5,022	0.81	MFOAD	0 – 2.4 % AR*	

[1] K_a = Adsorption coefficient
[2] K_{aOC} = Adsorption coefficient based on organic carbon content
[3] K_d = Desorption coefficient
[4] K_{dOC} = Desorption coefficient based on organic carbon content
[5] K_a / K_d = Adsorption/Desorption distribution coefficient
* = Results stated as % of applied radioactivity not % active substance

Mean adsorption values of 6,184 l kg^{-1} (Ka_{oc}) and 10,497 l kg^{-1} (Kd_{oc}) were derived from the soil study. These indicate that once adsorbed to the soil, Alc-*Z* is unlikely to be desorbed with water, and so suggest that Alc-*Z* exhibits a very low mobility potential in the four soils tested. This supports the K_{oc} values determined by the HPLC method.

In summary, the studies suggest Metofluthrin will have low mobility in soil, and a high potential to adsorb to suspended particulate matter in aquatic toxicity tests.

5.2.2 Volatilisation

The substance has a measured (DiFrancesco & Lentz, 2004a – A3 Doc IIIA) vapour pressure of 9.47 x 10^{-4} Pa at 20°C (98.4 % purity, RTZ: 86.6 %). The calculated Henry's Law constant is 0.681 Pa m^3/mole (Z isomer) and 0.509 Pa m^3/mole (E isomer) (DiFrancesco & Lentz, 2004b – A3 Doc IIIA). These values indicate limited partitioning from the aquatic environment to air.

5.2.3 Distribution modelling

Not relevant for this type of dossier.

5.3 Aquatic Bioaccumulation

Table 36: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
Method	**Results (l/kg)**	**Remarks**	**Reference**
QSAR	BCF = 3,548		Using Veith *et al*., 1979 EC, 2003

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

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

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

Using this equation, logBCF is 3.55, and the predicted BCF was 3, 548 l/kg. The QSAR is applicable to Metofluthrin as the equation is valid for substances with log K_{ow} between 2 and 6.

5.3.1.2 Measured bioaccumulation data

An aquatic fish bioconcentration study (Yakata, 2002- A7.4.3.3.1 doc IIIA) was conducted using the Common Carp (*Cyprinus carpio*) exposed to 99.2 % purity metofluthrin. The study was run according to OECD 305C (adopted 12 May 1981) and 'Methods for testing the degree of accumulation of chemical substances in fish body.' stipulated in 'Testing methods for new chemical substances.' (July 13, 1974, amended October 8, 1998; Kanpogyou no. 5, Yakuhatsu no. 615, 49-kikyoku no. 392). This was GLP compliant. The study was run for 60 days with exposure levels of 0.0005 mg/l and 0.00005 mg/l. The solutions were prepared using dispersant HCO-40 and a control. Both these concentrations are below the stated water solubility of the substance (0.50-0.67mg/l). In line with the Guideline applied at that time, no depuration phase was carried out.

Fish exhibited normal behaviour throughout the exposure and no mortalities were reported at any treatment. Concentrations of metofluthrin in water were well maintained; at steady-state they were 100 % and 94 % of nominal concentrations 0.0005 mg/l and 0.00005 mg/l, respectively, with mean concentrations after 60 days' exposure reported as 0.000486 and 0.0000451 mg/l. The mean fish

tissue concentrations after 60 days' exposure were 0.0553 and 0.0059 µg/g at the 0.0005 mg/l and 0.00005 mg/l test levels, respectively.

Measured BCFs were calculated on days 13, 25, 33, 47 and 60. The variation of mean BCF on days 33, 47 and 60 were within 20 % so it was considered that a steady state had been reached. Experimental steady-state BCF values were determined to be 110 and 120 l/kg at 0.0005 mg/l and 0.00005 mg/l exposure levels, respectively.

The fish lipid content was 3.61 % at the start of the study and 3.42 % at the end. BCFs were not lipid corrected in the study report or biocide CAR. The UK CA has used the arithmetic mean of these two values to calculate the lipid normalised (5%) BCF values. These are 156 and 171 for 0.0005 mg/l and 0.00005 mg/l exposure levels, respectively.

As no depuration phase was run, a DT_{50} in fish tissue for the active substance could not be established.

The isomeric purity was not known for the test substance used in the study. It is noted that the log Kow for both the E and the Z isomers is the same value.

5.3.2 Summary and discussion of aquatic bioaccumulation

The measured data are preferred to the predicted value, the difference presumably attributable to metabolism. The BCF of 156-171 l/kg suggests Metofluthrin is not significantly bioaccumulative.

5.4 Aquatic toxicity

The ecotoxicity tests were conducted on a test substance with >80% Z isomer. The toxicity of different isomers was not evaluated.

Table 37: 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 95.4%	Rainbow Trout (*Oncorhynchus mykiss*)	Mortality	*Flow-through*	96 hours	LC_{50}	0.0012(mm)	A7.4.1.1(01) Doc IIIA Lima W (2004)
Acute toxicity to fish OECD Guideline 203, GLP 94.9%	Common Carp (*Cyprinus carpio*)	Mortality	*Flow-through*	96 hours	LC_{50}	0.00306 (mm)	A7.4.1.1(02) Doc IIIA Gries, T (2002)
Daphnia sp Acute Immobilisation OECD Guideline, 202 GLP 95.4%	*Daphnia magna*	Acute immobilisation	*Flow-through*	*48 hours*	EC_{50}	0.0047 (mm)	A7.4.1.2 Doc IIIA Putt, A.E (2004)
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP 95.4%	*Pseudo-kirchneriella subcapitata*	Cell multiplication inhibition	*Static*	*72 hours*	ErC_{50} NOErC	0.37 0.11	*A7.4.1.3 Doc IIIA Hoberg, J. R (2004)*

mm refers to mean measured

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Two acute fish studies are available. Both were GLP compliant.

The first (Gries, 2002) was conducted on the Common Carp (*Cyprinus carpio*). This was a 96-hour flow-through study conducted according to OECD Guideline 203. A mixture of Alc-Z and Alc-E (88:12) diluted with non-labelled metofluthrin (94.9 % purity) were tested. The nominal concentrations were 0.000875, 0.00175, 0.0035, 0.007, 0.014 mg/l. Exposure solutions were prepared with the aid of toluene and dimethylformamide (DMF). The mean measured concentrations were 0.000712, 0.00154, 0.00353, 0.0068, 0.0126 mg/l. No lethal or sub-lethal effects were observed in the control or solvent control. A 96-h LC_{50} value of 0.00306 mg/l was reported for metofluthrin, based on mean measured concentrations. The 96-h NOEC was 0.000712 mg/l.

The second test (Lima, 2004) was undertaken using Rainbow Trout (*Oncorhynchus mykiss*). This was also a 96-hour flow-through study conducted according to OECD Guideline 203. The study used metofluthrin (95.4 % purity). The nominal concentrations were 0.00019, 0.00038, 0.00075, 0.0015, 0.003 mg/l. Exposure solutions were prepared with the aid DMF. The mean measured concentrations were 0.00019, 0.00034, 0.00071, 0.0015, 0.0026 mg/l. No lethal or sub-lethal effects were observed in the control or solvent control. The 96-h LC_{50} was 0.0012 mg/l based on mean measured concentrations. The 96-h NOEC was 0.00071 mg/l.

In both studies, the measured concentrations were > 80 % of the nominal concentrations.

5.4.1.2 Long-term toxicity to fish

No long-term tests are available.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

An acute 48-hour flow-through test (Putt, 2004) was performed with the water flea (*Daphnia magna*) using 95.4 % purity metofluthrin. This was run according to both US and OECD 202, and was to GLP. The nominal concentrations were 0.00075, 0.0015, 0.003, 0.006 and 0.012 mg/l. Exposure solutions were prepared with the aid of DMF. Analysis indicated measured concentrations at the end of the test were > 80 % of the nominal concentrations. The mean measured concentrations were 0.00085, 0.0016, 0.003, 0.0058 and 0.011 mg/l. No lethal or sub-lethal effects were observed in the control or solvent control. A 48-h EC_{50} value of 0.0047 mg/l was reported, based on mean measured concentrations. The 48-h NOEC was 0.003 mg/l.

5.4.2.2 Long-term toxicity to aquatic invertebrates

No long-term tests are available.

5.4.3 Algae and aquatic plants

A 72-hour growth inhibition test (Hoberg, 2004) using metofluthrin (95.4 % purity), was performed using the freshwater green alga *Pseudokirchneriella subcapitata* according to OECD Guideline 201. The study was GLP compliant. Nominal concentrations prepared with DMF were 0.0088, 0.019, 0.043, 0.094, 0.21, 0.45 and 1.0 mg/l. At 72 hours cells in all control and exposure treatments were observed to be normal. No statistical difference was observed between controls and solvent controls and all validation criteria were met. Measured test concentrations were 48-100 % of nominal at the start of the test, and 38-68 % of nominal after 72 hours. The poor level of concentration maintenance was thought to be due to the limited solubility of metofluthrin in the algal medium, and the results were therefore reported as the mean measured concentrations. The 72-h E_rC_{50} was 0.37 mg/l and 72-h NOE_rC value was 0.11 mg/l.

5.4.4 Other aquatic organisms (including sediment)

No sediment studies using aquatic exposure are available.

An activated sludge respiration inhibition test (McLaughlin, 2004 – A7.4.1.4 Doc IIIA) was conducted according to OECD Guideline 209. It is reported here as no toxicity control was included in the ready biodegradation test. A preliminary test was conducted using concentrations of 0.1, 1, 10, 100 and 1,000 mg/l of metofluthrin (95.4 % purity). This showed that, compared with the controls, respiratory inhibition was 0, 4.5, 0, 0 and 0 %, respectively. As the highest preliminary test concentration produced no inhibition of micro-organism respiration within the sludge, it was not necessary to conduct a full test. The 3-h EC_{10} was therefore considered to be 1,000 mg/l of metofluthrin, and the 3-h EC_{50} was > 1,000 mg/l. The study was GLP compliant.

There are no aquatic toxicity data available for the metabolites of Metofluthrin.

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

Metofluthrin is not readily biodegradable following an OECD 301C study. In the environment it is generally hydrolytically stable, although slight hydrolysis will occur at pH 9. Overall, Metofluthrin is considered not rapidly (or readily) degradable for the purposes of hazard classification.

The measured BCF value for Metofluthrin is below 500.

The ecotoxicity test results show that Metofluthrin exhibits a similar level of acute aquatic toxicity to both fish and invertebrates, with algae being significantly less sensitive. The most sensitive endpoint is the fish 96-h LC_{50} of 0.0012 mg/l (mean measured concentrations). The only chronic data point is the 72-h NOEC from the algal test. As this taxanomic group is clearly less sensitive than fish or invertebrates in acute tests, this means there are insufficient chronic data for classification. In this case the surrogate approach using the acute data should be used.

Based on acute aquatic toxicity data with short-term $L(E)C_{50}$ values below 1 mg/l, Metofluthrin should be classified as Aquatic Acute 1. An acute M-factor of 100 is applicable based on $0.001 < L(E)C_{50} \leq 0.01$ mg/l.

Adequate chronic toxicity data are not available. Using the surrogate approach Metofluthrin should be classified Aquatic Chronic 1, as the short-term $L(E)C_{50}$ values are below 1 mg/l and it is not rapidly degradable. A chronic M-factor of 100 is applicable.

5.6 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

6 OTHER INFORMATION

None

7 REFERENCES

1. Competent Authority Report (CAR) – Document IIA – Effects Assessment for the Active Substance – Metofluthrin – June 2010
2. Competent Authority Report (CAR) – Document IIIA – Effects Assessment for the Active Substance – Metofluthrin – June 2010
3. EC(2003) Technical Guidance Document (TGD) on Risk Assessment

Author(s)	Year	Title Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published
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8 ANNEXES

Annex I; An Evaluation of the Human Relevance of Metofluthrin-induced Liver Tumours in Rats Based on Mode of Action – Yamada - 2012