Committee for Risk Assessment

RAC

Annex 1

Background document

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

(1,3,4,5,6,7-hexahydro-1,3-dioxo-2H-isoindol-2-yl)methyl (1R-trans)-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate

EC Number: 214-619-0
CAS Number: 1166-46-7

CLH-O-0000001412-86-126/F

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

Adopted

16 September 2016

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

(1,3,4,5,6,7-hexahydro-1,3-dioxo-2*H*-isoindol-2-yl)methyl (1R-*trans*)-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate; d-*trans*-tetramethrin

EC Number: **214-619-0**

CAS Number: **1166-46-7**

Index Number: **-**

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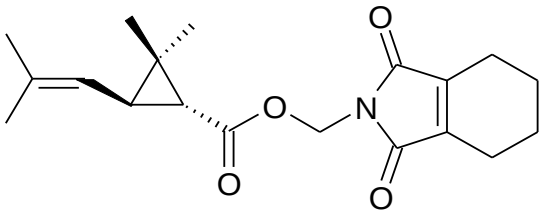
CONTENTS

1. IDENTITY OF THE SUBSTANCE

1.1 NAME AND OTHER IDENTIFIERS OF THE SUBSTANCE

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

Name(s) in the IUPAC nomenclature	(1,3-dioxo-1,3,4,5,6,7-hexahydro-2*H*-isoindol-2-yl)methyl (1R,3R)-2,2-dimethyl-3-(2-methylprop-1-en-1-yl)cyclopropanecarboxylate
Other names (usual name, trade name, abbreviation)	d-*trans*-Tetramethrin; CAS name: Cyclopropanecarboxylic acid, 2, 2-dimethyl-3-(2-methyl-1-propen-1-yl)-, (1, 3, 4, 5, 6, 7- hexahydro-1, 3- dioxo-2*H*-isoindol-2-yl) methyl ester, (1R, 3R)-
EC number (if available and appropriate)	214-619-0
EC name (if available and appropriate)	(1,3,4,5,6,7-hexahydro-1,3-dioxo-2*H*-isoindol-2-yl)methyl (1R-*trans*)-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate
CAS number (if available)	1166-46-7
Molecular formula	$C_{19}H_{25}NO_4$
Structural formula	
Molecular weight or molecular weight range	331.41 g/mol

1.2 COMPOSITION OF THE SUBSTANCE

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
(1,3-dioxo-1,3,4,5,6,7-hexahydro-2*H*-isoindol-2-yl)methyl (1*R*,3*R*)-2,2-dimethyl-3-(2-methylprop-1-en-1-yl)cyclopropanecarboxylate; EC number: 214-619-0			

For further information: Please refer to the confidential annex.

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

Impurity (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The impurity contributes to the classification and labelling**
For further information: Please refer to the confidential annex.				

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

Additive (Name and numerical identifier)	**Function**	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The additive contributes to the classification and labelling**
For further information: Please refer to the confidential annex.					

Table 5: Test substances (non-confidential information)

Identification of test substance	**Purity**	**Impurities and additives (identity, %, classification if available)**	**Other information**

2. PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 PROPOSED HARMONISED CLASSIFICATION AND LABELLING ACCORDING TO THE CLP CRITERIA

Table 6: Proposed harmonised classification and labelling according to the CLP criteria

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	-										
Dossier submitters proposal	607-RST-00-Y	(1,3,4,5,6,7-hexahydro-1,3-dioxo-2H-isoindol-2-yl)methyl (1R-trans)-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane carboxylate; d-*trans*-tetramethrin	214-619-0	1166-46-7	Acute Tox. 4 Carc. 2 STOT SE 2 Aquatic Acute 1 Aquatic Chronic 1	H332 H351 H371 H400 H410	GHS07 GHS08 GHS09 Warning	H332 H351 H371 H410		M = 100 (acute) M = 100 (chronic)	
Resulting Annex VI entry if agreed by RAC and COM	607-RST-00-Y	(1,3,4,5,6,7-hexahydro-1,3-dioxo-2H-isoindol-2-yl)methyl (1R-trans)-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane carboxylate; d-*trans*-tetramethrin	214-619-0	1166-46-7	Acute Tox. 4 Carc. 2 STOT SE 2 Aquatic Acute 1 Aquatic Chronic 1	H332 H351 H371 H400 H410	GHS07 GHS08 GHS09 Warning	H332 H351 H371 H410		M = 100 (acute) M = 100 (chronic)	

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

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

RAC general comment

Tetramethrin is a biocide and a plant protection product with no current entry in Annex VI. The dossier submitter (DS) has presented studies for assessing all human health hazards using tetramethrin, although there is also a large database of information with studies performed with d-trans-tetramethrin ((1,3,4,5,6,7-hexahydro-1,3-dioxo-2H-isoindol-2-yl)methyl (1R-trans)-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate) that serve as additional key information.

Justification for read-across between tetramethrin and d-trans-tetramethrin

Tetramethrins are esters of chrysanthemic acid with 3,4,5,6-tetrahydrophthalimidomethyl alcohol and are classified as type I pyrethroids, which lack a cyano group within the alcohol moiety. Tetramethrin and d-trans-tetramethrin are isomeric mixtures of [1R, cis], [1S, cis], [1R, trans] and [1S, trans]-tetramethrin, differing in the ratios of individual stereoisomers. The table below shows the average isomeric composition of 5 batches of the technical products tetramethrin and d-trans-tetramethrin as well as the range of isomeric composition derived from four batches of tetramethrin. [1R, trans] isomer prevailed in the composition of both tetramethrins, representing more than 90% in the case of d-trans-tetramethrin. This [1R, trans] isomer displays the highest potential for causing impairments in the normal working of the axonal sodium channels (the main mechanisms for its biocidal activity). The toxic potency of individual isomers in mammals has not been defined.

Table: Average of isomeric composition of technical products containing d-trans-tetramethrin and tetramethrin

Product	Batches	[1R, cis] (%)	[1S, cis] (%)	[1R, trans] (%)	[1S, trans] (%)
d-trans-tetramethrin (Sumitomo)	5	2.8	0.2	93.1	3.9
Tetramethrin (Sumitomo)	5	10.1	10.0	39.7	40.2
Tetramethrin (Endura)	4	9.1-10.3	7.6-10.3	40.0-46.9	35.5-40.4

As seen in the table below, a complete set of data for human health is available only for tetramethrin, although carcinogenicity is the only hazard that has not been assayed for d-trans-tetramethrin. It can also be noted that the number of independent studies is, in general, higher for tetramethrin than for d-trans-tetramethrin.

Table: Hazard-specific studies using tetramethrin and d-trans-tetramethrin.

Endpoint/hazard class	Tetramethrin	d-trans-tetramethrin	DS proposal
Physico-chemical properties	X	No data	No classification and labelling
Acute toxicity (oral)	X	X	No classification and labelling
Acute toxicity (dermal)	X	X	No classification and labelling

Acute toxicity (inhalation)	X	X	Category 4 (H332)
STOT SE			Category 2 (H371)
Skin irritation	X	X	No classification and labelling
Eye Irritation	X	X	No classification and labelling
Skin sensitisation	X	X	No classification and labelling
Respiratory sensitisation	X (epidemiological)	No data	No classification and labelling
STOT RE	X	X	No classification and labelling
Mutagenicity *(in vitro)*	X	X	No classification and labelling
Mutagenicity *(in vivo)*	X	X	No classification and labelling
Carcinogenicity	X	No data	Category 2 (H371)
Fertility and sexual function	X	X	No classification and labelling
Development	X	X	No classification and labelling

The comparison of the available data base appears to scientifically support that data of each of the compounds may be used to predict toxicity and fate of the other on the basis of the following facts:

1. The two substances (tetramethrin and d-trans-tetramethrin) are chemical isomers and therefore have the same physical properties;

2. Symptoms of neurotoxicity were observed for both tetramethrins as a result of both acute and repeated inhalative exposure; this has been typically reported for other type I pyrethroids as well;

3. Very low acute toxicity was observed for both substances after acute oral and dermal exposure;

4. The NOAECs for neurotoxicity reported for both substances were within the same order of magnitude (0.044 mg d-trans-tetramethrin/L/3 hours/d with 28 days of exposure and 0.02 mg tetramethrin/L/4 hours/d with 90 days of exposure; both in rat);

5. In addition to the above stated neurotoxic effects, the other relevant effect detected after repeated exposure were liver, haematology and clinical chemistry alterations reported for both d-trans-tetramethrin and tetramethrin;

6. NOAEL/LOAEL intervals for d-trans-tetramethrin and tetramethrin in medium- and long-term studies are comparable and overlapping (see table below); which suggests similar potency regarding liver toxicity;

Table: NOAEL/LOAEL of tetramethrin and d-trans-tetramethrin in repeated toxicity studies by oral route.

Species	Duration	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)
tetramethrin			
Rat	90 days	76	151
Rat	6 month	95	325
Dog	6 month	90	180

d-trans-tetramethrin			
Rat	28 days	290 (M)/ 295 (F)	965 (M) /940 (F)
Rat	6 month	58 (M) /71 (F)	178 (M) /214 (F)

7. No evidence for toxic effects on foetuses below doses causing maternal toxicity were seen in developmental toxicity studies for either d-trans-tetramethrin or tetramethrin;

8. Neither d-trans-tetramethrin nor tetramethrin met the criteria for classification as skin or eye irritating or as genotoxic;

9. Neither of the substances caused sensitisation in Guinea pig (Buehler) tests.

In conclusion, **RAC agreed with the DS that data from tetramethrin can be used as additional evidence for the assessment of human health hazards of d-trans-tetramethrin.**

3. HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

4. JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

The substance is an active substance in the meaning of Directive 91/414/EEC (repealed by the Regulation EC 1107/2009) or Directive 98/8/EC (will be repealed by Regulation (EU) No 528/2012 on 1 September 2013) shall normally be subject to harmonised classification and labelling, and justification is not required. (Article 36 CLP Regulation)

There is no requirement for justification that action is needed at Community level.

5. IDENTIFIED USES

6. DATA SOURCES

7. PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	extremely viscous liquid (T = 25 °C) *(88.7% 1R-trans-Isomer)*	O'Donnell, R. T. et al Sumitomo Report No. SVP-0008, 1999	measured *(visual assessment)*
Melting/freezing point	19 °C ± 3 °C (pour point) *(89.5% 1R-trans- Isomer)*	Evans A.J. et al, SPL Project No: 1430/011, 2002	measured *(92/69/EEC, A.1; pour point)*
Boiling point	263 °C (pressure**:** 988 hPa) *(100%,* 1R-trans-Isomer*)*	Malinkski, M. F. Sumitomo Report No. SVP-0003, 1999	measured *(92/69/EEC, A.2 ; DSC)*
Relative density	1.11 g/cm^3 *(92.1% 1R-trans-Isomer)*	Lentz NR (2008), Sumitomo Report No SVP-0020	measured *(92/69/EEC, A.3; Pycnometer method)*
Vapour pressure	4.03*10^{-6} Pa (T = 25 °C), 1.56 * 10^{-6} Pa (T = 20 °C) *(100%, 1R-trans-Isomer)*	Schetter, J. E. Sumitomo Report No. SVP-0006, 1999	measured *(92/69/EEC, A.4; gas saturation method)*
Surface tension	55.9 mN/m (mean) (T = 20.4 °C, c = 1.58 mg/l) *(92.1 % 1R-trans-Isomer)*	Lentz NR (2008), Study No. 13048.6593	measured *(92/69/EEC, A.5; ring method)*
Water solubility	1.60 ± 0.21 mg/l (mean) at T = 20 °C, pH = 6 (unbuffered) *(92.1 % 1R-trans-Isomer)*	Lentz NR (2008), Study No. 13048.6571	measured *(92/69/EEC, A.6; column elution method)*
Partition coefficient n-octanol/water	log Pow = 4.3 (T = 25 °C) *(100%, 1R-trans-Isomer)*	Dudones, L. P. Sumitomo Report No. SVP-0004 (1999)	measured *(92/69/EEC, A.8; HPLC method)*

Flash point	148 °C (± 2 °C) (Purity: 96.0 %)	White D. F., Mullee D. M., SPL Project No: 0555/0076, 2006	measured (92/69/EEC, A.9: ISO 3679:1983 Setaflash closed-cup apparatus)
Flammability	Since the flash point for d-tetramethrin was found to be 148 ± 2 °C, a flammability study is scientifically unjustified. Flammability in contact with water: The classification procedure needs not to be applied because the organic substance does not contain metals or metalloids. Pyrophoric properties: The classification procedure needs not to be applied because the organic substance is known to be stable into contact with air at room temperature for prolonged periods of time (days).	Sumitomo Chemical (2006) BAM 2.2 (2014)	Justification for non-submission of data - Method 92/69/EEC, A.10, A.11 Justification for non-submission of data - Method 92/69/EEC, A.12, A.13
Explosive properties	d-Tetramethrin does not have any functional groups such as diazo, azide, polynitro or peroxide, which are found in chemically explosive compounds. Therefore, chemical explosion reactions are considered not to occur under ordinary circumstances. A study to determine explosive properties would therefore be scientifically unjustified.	Sumitomo Chemical (2006)	Justification for non-submission of data - Method 92/69/EEC, A.14
Self-ignition temperature	366 °C (± 5°C) (atmospheric pressure: 101.25 to 102.73 kPa) (Purity: 96.0 %)	White D.F., ., Mullee D. M., SPL Project No: 0555/0076, 2006	measured (92/69/EEC, A.15)
Oxidising properties	d-Tetramethrin does not have any functional groups associated with oxidising action. It does not undergo substitution, addition or elimination reactions. Therefore, oxidising action is considered not to occur under ordinary circumstances. A study to determine oxidising properties would therefore be scientifically unjustified.	Sumitomo Chemical (2006)	Justification for non-submission of data - Method 92/69/EEC, A.21

Granulometry	Not applicable. *(The substance is a liquid under ambient conditions.)*	-	-
Stability in organic solvents and identity of relevant degradation products	The test substance is stable at T = 40 °C for 3 month in the following solvents: Xylene, Deobase, Methyl isobutyl Ketone, Ethyl acetate, Acetonitrile, Chloroform, Propionic Acid, Dimethylformamide, Isopropyl alcohol, Kerosene, Methyl Chlorofom The test substance is not stable at T = 60 °C for 3 month in the following solvents: Methanol, Ethylcellosorb, Cyclohexanone, Chloroform, Propionic acid, Dimethylformamide, Corn oil *(74.9 %, 1R-trans-Isomer, 17.5 % 1R-cis-Isomer)*	Sumitomo Chemical Co., Sumitomo Report No. IP-10-0054, 1986	measured *(Gaschromatography)*
Dissociation constant	*pKa = -2.55 ± 0.20 (calculation)*	Roth H., Safepharm Laboratories, 2006	*calculated*
Viscosity	8.89 x 10^4 mPa.s temperature: 20.0°C *(89.7 % 1R-trans-Isomer)*	White D.F., SPL Project No: 0555/0076, 2006	measured *(OECD 114; rotational viscometer)*

8. EVALUATION OF PHYSICAL HAZARDS

8.1 EXPLOSIVES

Table 9: Summary table of studies on explosives

Method	Results	Remarks	Reference
Justification for data waiving	d-Tetramethrin does not have any functional groups such as diazo, azide, polynitro or peroxide, which are found in chemically explosive compounds. Therefore, chemical explosion reactions are considered not to occur under ordinary circumstances. A study to determine explosive properties would therefore be scientifically unjustified.		Sumitomo Chemical (2006)

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

Justification is acceptable because there are no chemical groups present in the molecule which are associated with explosive properties.

8.1.2 Comparison with the CLP criteria

The classification procedure needs not to be applied due to justification for data waiving.

8.1.3 Conclusion on classification and labelling for explosive properties

Should not be classified as explosives according to Annex I part 2 of the CLP regulation.

8.2 FLAMMABLE GASES (INCLUDING CHEMICALLY UNSTABLE GASES)

Hazard class not applicable.

8.3 OXIDISING GASES

Hazard class not applicable.

8.4 GASES UNDER PRESSURE

Hazard class not applicable.

8.5 FLAMMABLE LIQUIDS

Table 10: Summary table of studies on flammable liquids

Method	Results	Remarks	Reference
92/69/EEC, A.9: ISO 3679:1983 Setaflash closed-cup apparatus	148 °C (± 2 °C)		White D. F., Mullee D. M.,SPL Project No: 0555/0076, 2006

8.5.1 Short summary and overall relevance of the provided information on flammable liquids

A single study is available. The flash point for d-tetramethrin was found to be 148 ± 2 °C.

8.5.2 Comparison with the CLP criteria

A test was conducted according to EU Method A.9. Based on the experimental data it is concluded that d-tetramethrin with a flash point of more than 60 °C does not meet the criteria for classification as flammable liquid according to Annex I part 2 of the CLP regulation.

8.5.3 Conclusion on classification and labelling for flammable liquids

Should not be classified as flammable liquid according to Annex I part 2 of the CLP regulation.

8.6 FLAMMABLE SOLIDS

Hazard class not applicable.

8.7 SELF-REACTIVE SUBSTANCES

Table 11: Summary table of studies on self-reactivity

Method	Results	Remarks	Reference
Justification for data waiving	The classification procedure needs not to be applied because there are no chemical groups present in the molecule which are associated with explosive or self-reactive properties.		BAM 2.2 (2014)

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

Justification is acceptable.

8.7.2 Comparison with the CLP criteria

The classification procedure needs not to be applied due to justification for data waiving.

8.7.3 Conclusion on classification and labelling for self-reactive substances

Should not be classified as self-reactive substance according to Annex I part 2 of the CLP regulation.

8.8 PYROPHORIC LIQUIDS

Table 12: Summary table of studies on pyrophoric liquids

Method	Results	Remarks	Reference
Justification for data waiving	The classification procedure needs not to be applied because the organic substance is known to be stable into contact with air at room temperature for prolonged periods of time (days).		BAM 2.2 (2014)

8.8.1 Short summary and overall relevance of the provided information on pyrophoric liquids

Justification is acceptable.

8.8.2 Comparison with the CLP criteria

The classification procedure needs not to be applied due to justification for data waiving.

8.8.3 Conclusion on classification and labelling for pyrophoric liquids

Should not be classified as pyrophoric liquid according to Annex I part 2 of the CLP regulation.

8.9 PYROPHORIC SOLIDS

Hazard class not applicable.

8.10 SELF-HEATING SUBSTANCES

Hazard class not applicable.

8.11 SUBSTANCES WHICH IN CONTACT WITH WATER EMIT FLAMMABLE GASES

Table 13: Summary table of studies on substances which in contact with water emit flammable gases

Method	Results	Remarks	Reference
Justification for data waiving	The classification procedure needs not to be applied because the organic substance does not contain metals or metalloids.		BAM 2.2 (2014)

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

Justification is acceptable.

8.11.2 Comparison with the CLP criteria

The classification procedure needs not to be applied due to justification for data waiving.

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

Should not be classified as substance which in contact with water emits flammable gases according to Annex I part 2 of the CLP regulation.

8.12 OXIDISING LIQUIDS

Table 14: Summary table of studies on oxidising liquids

Method	Results	Remarks	Reference
Justification for data waiving	d-Tetramethrin does not have any functional groups associated with oxidising action. It does not undergo substitution, addition or elimination reactions. Therefore, oxidising action is considered not to occur under ordinary circumstances. A study to determine oxidising properties would therefore be scientifically unjustified.		Sumitomo Chemical (2006)

8.12.1 Short summary and overall relevance of the provided information on oxidising liquids

Justification is acceptable because there are no chemical groups present in the molecule which are associated with oxidising properties.

8.12.2 Comparison with the CLP criteria

The classification procedure needs not to be applied due to justification for data waiving.

8.12.3 Conclusion on classification and labelling for oxidising liquids

Should not be classified as oxidising liquid according to Annex I part 2 of the CLP regulation.

8.13 OXIDISING SOLIDS

Hazard class not applicable.

8.14 ORGANIC PEROXIDES

Hazard class not applicable.

8.15 CORROSIVE TO METALS

Hazard class not assessed in this dossier.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The DS reported that explosive, flammability and oxidising properties have been tested with results not warranting classification. Taking into consideration the chemical structure of d-trans-tetramethrin, all other relevant physico-chemical parameters can be waived. The DS proposed no classification in relation to physico-chemical hazards.

Comments received during public consultation

One Member State Competent Authority (MSCA) supported the no classification proposal.

Assessment and comparison with the classification criteria

Tetramethrin did not exhibit explosive properties in a normalised EU A.14 assay. In addition tetramethrin does not have any functional groups, such as diazo, azide, polynitro or peroxide, typically found in chemically explosive compounds.

D-trans-tetramethrin showed a flash point of 148 ± 2 °C in a normalised EU A.9 assay and therefore does not meet the criteria for classification as flammable liquid since this is higher than 60 °C.

The classification for self-reactive substances does not need to be applied because there are no chemical groups present in the molecule associated with explosive or self-reactive properties.

The classification for pyrophoric solids does not need to be applied because the organic substance is known to be stable in contact with air at room temperature for prolonged periods of time (days).

D-trans-tetramethrin does not contain metals or metalloids, hence no test was needed to measure emission of flammable gases when the substance is in contact with water.

D-trans-tetramethrin does not have any functional groups associated with oxidising action. It does not undergo substitution, addition or elimination reactions. Therefore, oxidising action is considered not to occur under ordinary circumstances. In addition, according to guidelines the solid organic substances containing oxygen chemically bonded only to carbon or hydrogen (as is the case of d-trans-tetramethrin) do not need to be classified as oxidising.

The hazard 'corrosivity to metals' has not been assessed in the CLH dossier. Nevertheless, the absence of acidic or basic functional groups and immiscibility with water suggests that the substance is, most likely, not corrosive to metals.

In conclusion, RAC agreed with the DS's proposal for **no classification** of d-trans-tetramethrin **regarding physico-chemical hazards.**

9. TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 15: Summary table of toxicokinetic studies

Method, guideline	Species, strain, sex, no/group	Test substance, reference to table 5	Dose levels, duration of exposure	Results	Remarks	Reference
Similar to OECD 417, oral, gavage	Rat, Sprague-Dawley, Low, high and repeated dose groups: 5 M + 5 F Controls: 3 M + 3 F	[1RS, trans]-tetramethrin in corn oil, C-14-labelled and unlabelled	Low single dose group with labelled material: 2 mg/kg bw High single dose group with labelled material: 250 mg/kg bw Repeated dose group: 14 days pre-treatment with unlabelled material at 2 mg/kg bw/day, followed by single dose of labelled material (2 mg/kg bw) Control group: 14 days pre-treatment with corn oil at 5 ml/kg/day, followed by single dose of labelled material (2 mg/kg bw)	Radiocarbon absorption 42-71 % (based on C-14 excretion in urine), 94-100 % and 95-101 % excretion of radioactivity within 2 and 7 days, respectively (urine: 42.3-71.4 %, faeces: 29-57.9 %, air: < 0.1 %), 0.2-0.4 % C-14 tissue residues after 7 days, widely distributed, highest residues in blood cells Excreted at >5 % in urine plus faeces within 2 days after dosing of radioactive compound in any group: 1-sulfo-cyclohexane-dicarboximide, 3-hydroxy-cyclohexane-dicarboximide Excreted unmetabolised parent in urine plus faeces: 5-23 % in single dose groups, < 3 % in repeated dose groups	C-14 label at phthalimide moiety only (alcohol label) 48 % of radioactivity in unknown or non-extractable metabolites	Shiba K, 1992, Sumitomo Report No. IM-20-0015 Parts of study also published in: Tomigahara et al., 1994, Xenobiotica 24(12): 1205-1214

Method, guideline	Species, strain, sex, no/group	Test substance, reference to table 5	Dose levels, duration of exposure	Results	Remarks	Reference
Sim. to OECD 417, Oral, gavage	Rat, Sprague-Dawley, Low, high and repeated dose groups: 5 M + 5 F Controls: 3 M + 3 F	[1RS, cis]-tetramethrin in corn oil, C-14-labelled and unlabelled	Low single dose group with labelled material: 2 mg/kg bw High single dose group with labelled material: 250 mg/kg bw Repeated dose group: 14 days pre-treatment with unlabelled material at 2 mg/kg bw/day, followed by single dose of labelled material (2 mg/kg bw) Control group: 14 days pre-treatment with corn oil at 5 ml/kg/day, followed by single dose of labelled material (2 mg/kg bw)	Radiocarbon absorption 9-32 % (based on C-14 excretion in urine), 95-101 % and 96-102 % excretion of radioactivity within 2 and 7 days, respectively (urine: 8.5-32.4, faeces: 65.9-91.3, air: < 0.1 %), 0.2-0.4 % C-14 tissue residues after 7 days, widely distributed, highest residues in blood cells Excreted at > 5 % in urine plus faeces within 2 days after dosing of radioactive compound in any group: 3-hydroxy-cyclohexane-dicarboximide, 1-sulfo-cyclohexane-dicarboximide, N-(hydroxymethyl)-3-hydroxy-1-sulfo-cyclohexane-dicaboximide, unknown compounds: 30, 34 and 39 Excreted unmetabolised parent in urine plus faeces: 13-34 % in single dose groups, < 4 % in repeated dose groups	C-14 label at phthalimide moiety only (alcohol label) 42-68 % unknown, non-extractable or other radioactive metabolites	Shiba K, 1992, Sumitomo Report No. IM-20-0016 Parts of study also published in: Tomigahara et al., 1994, Xenobiotica 24(12): 1205-1214

Method, guideline	Species, strain, sex, no/group	Test substance, reference to table 5	Dose levels, duration of exposure	Results	Remarks	Reference
Supplementary information Non guideline, non-GLP Oral and subcutaneous	Rat, Sprague-Dawley, 3-4 M + 3-4 F	C-14 [1R, trans]- and [1R, cis]-tetramethrin, respectively, each preparation suspended in 10% Tween 80	Single dose: 3.2 to 5.3 mg/kg bw	27-46 and 42-67 % radiocarbon absorption after oral administration of [1R, cis]- and [1R, trans]-isomer (urinary C-14 excretion); > 90 % C-14 excretion within 7 d of oral or subcutaneous administration (cis/trans in urine: 27-49/42-74 %, faeces: 45-68/21-55 %, air: < 1 - 3 %); 2-3-fold slower initial metabolism and C-14 elimination after s.c. application of the cis-isomer as compared to the trans-isomer, ~5-fold lower C-14 residues (day 7) for acid than alcohol labelled tetramethrin isomers	C-14 label at phthalimide (alcohol) or chrysanthemic acid moiety	Sumitomo Report No: IM-10-0006 Summary of study published: Kaneko H, Ohkawa H, Miyamoto J, 1981, J. Pesticide Sci. 6 (4): 425-435
Supplementary information Non guideline, non-GLP *In vitro* (rat liver microso-mes)	Rat, Sprague-Dawley, male, (no. not specified, for microsome preparation only)	[1RS, trans]-tetramethrin	1 mM (331 ppm), C-14 [RS, trans]-tetramethrin, 1 hour incubation (37 °C)	68.0/58.5 % of acid/ alcohol labelled substance degraded within 1 h by 7 mg protein eq. of rat liver microsomes in the presence of NADPH, ~50 % NADPH-dependent oxidation, ~50 % paraoxon-sensitive ester hydrolysis, non-enzymatic decomposition of N-(hydroxymethyl)-tetrahydrophthalimide to tetrahydrophthalimide	C-14 [1RS, trans]-tetramethrin, labelled at alcohol or acid moiety: Alcohol labelled: ^{14}C at the carbonyl group in the tetrahydrophthalimide moiety Acid labelled: ^{14}C at the carboxyl position in the chrysanthemumic acid moiety	Suzuki T, Miyamoto J, 1974, Pesticide Biochem. Physiol. 4(1): 86-97
Supplementary information	Rat, Wistar, Male,	C-14 [1RS, trans]-tetramethr	Single dose: 1.5 (i.v.) or 500 (oral) mg/kg bw	Oral radiocarbon absorption approx. 50 % (based on metabolites in urine);	C-14 label at phthalimide moiety (alcohol label)	Miyamoto J et al., 1968, Agr. Biol. Chem. 32(5): 628-640

Method, guideline	Species, strain, sex, no/group	Test substance, reference to table 5	Dose levels, duration of exposure	Results	Remarks	Reference
Non guideline, non-GLP, oral and i.v.	(no. not specified)	in emulsion,		~90 and > 95 % radiocarbon excretion within 2 and 5 d after oral admin., respectively (urine: 42.3-71.4, faeces: 29-57.9, air: < 0.1 %); sustained oral C-14 absorption with $t_{1/2}$ of ~ 2 h, rapid systemic degradation of parent compound with $t_{1/2}$ of ~ 10 min (i.v.), limiting the percentage of parent compound to < 1 % of C-14 from 1 h after oral administration		Sumitomo Report No: IM-80-0003
Supplementary information Non guideline, non-GLP i. p.	Mice, male albino, Rat, male albino (no. not specified)	C-14 tetramethrin	Single dose: 30µl/20 g mouse 150 µl/200 g rat	Tetramethrin underwent Michael addition with thiols, tetramethrin-gluthathione conjugate is formed in the presence of mouse liver homogenate, mercapturic acid or tetramethrin-gluthathion conjugates were not found in bile or urine of rats or mice, biliary excretion of C-14 tetramethrin: 6% in 2 h and 51% in 24 h 50-66 % urinary excretion of C-14 tetramethrin within 24 h	C-14 label at acid or alcohol moiety	Smith, I.H., Wood, E.J., & Casida, J.E., 1982,. J. agri. food Chem. 30: 598-600 (not submitted by applicant)
Dermal absorption study *in vitro* OECD 428	Heat separated human epidermis, *in vitro*	Tetramethrin, dissolved in ethanol	20 µL/cm^2 (200 µg/cm^2 in ethanol), 24 h	22.3 µg/cm^2 (11.2 % of dose) absorbed		Hadfield N, 2006, Central Toxicology Laboratory Report No. JV1900; Sumitomo
Dermal absorption study *in vitro*	Heat separated human	d-Tetramethrin,	20 µL/cm^2 (200 µg/cm^2 in ethanol), 24 h	12.4 µg/cm^2 (6.2 % of dose) absorbed		Hadfield N, 2006, Central Toxicology Laboratory

Method, guideline	Species, strain, sex, no/group	Test sub-stance, reference to table 5	Dose levels, duration of exposure	Results	Remarks	Reference
OECD 428	epidermis, *in vitro*	dissolved in ethanol				Report No. JV1901; Sumitomo

9.1 SHORT SUMMARY AND OVERALL RELEVANCE OF THE PROVIDED TOXICOKINETIC INFORMATION ON THE PROPOSED CLASSIFICATION(S)

A number of studies have been performed in the rat to address the toxicokinetics of tetramethrins using [1RS, trans]-, [1RS, cis]-, [1R, trans] and [1R, cis]-isomers labelled at their alcohol (phthalimide) or acid (chrysanthemic acid) moiety. From the absence of detectable differences between the 1RS- and the 1R-isomers, it may be concluded that the general toxicokinetic behaviour of the 1R- and the 1S-isomers is similar. Minor differences relating to the relative amount of radiocarbon metabolites excreted via urine and the initial elimination velocity were observed for the trans- and cis-isomers (Kaneko et al., 1981; Sumitomo). Considering the low content of cis-isomers of 20 % and 3 % in technical products of tetramethrin and d-tetramethrin, respectively, these minor differences appear not to be of significant practical relevance.

Radiocarbon absorption after administration of acid- and alcohol-labelled C-14 tetramethrin may be estimated from the relative amount excreted in urine and amounted to 42-74 and 9-46 % of trans- and cis-isomers, respectively, depending on dose and pre-treatment. Considering the prevalence of the trans-isomers in tetramethrin technical products (see Table 3-1), the oral absorption is estimated to be 50 % of C-14 tetramethrin.

Radioactivity in blood and tissues suggests sustained absorption of orally administered tetramethrin isomers with an estimated absorption half-life of approx. 2 h. In the body tetramethrin is degraded very rapidly as indicated by a t_{max} of 1 h for the parent compound compared to 8 h for radiocarbon. Analysis of blood levels of the parent compound and metabolites following i.v. administration support rapid degradation with a $t_{1/2}$ of approx. 10 min. When administered orally, the percentage of undegraded a.s. was less than 1 % of total radiocarbon in blood as early as 1 hour following administration, although it remained unclear whether the responsible metabolic reactions occurred systemically or pre-systemically (Miyamoto et al., 1968).

The bioavailability of orally administered (d-)tetramethrin for the systemic circulation as undegraded substance presents a very rough estimate of 0.5 % to 50 %. Consequently, internal reference doses are extrapolated from inhalation rather than from oral studies.

Total excretion of orally or subcutaneously administered radiocarbon was generally ≥ 90 % after 2 days and ≥ 95 % after 1 week. Detectable amounts in exhaled air were reported for acid-labelled tetramethrin only: up to 3 % of the orally (but not subcutaneously) administered dose of cis- as well as trans-isomers were exhaled as CO_2 (Kaneko et al., 1981; Sumitomo). Remaining radioactive tissue residues 5-7 days after dosing were generally low with 0.2-0.4 % and widely distributed. Highest residue concentrations were found in blood cells. Residues after administration of acid-labelled tetramethrins were approx. 5 times lower than for alcohol-labelled isomers (Kaneko et al., 1981).

Initial metabolism and elimination of the cis-isomers after subcutaneous administration was 2-3 times slower than for the trans-isomers (Kaneko et al., 1981; Sumitomo).

Main metabolic reactions include ester hydrolysis yielding chrysanthemic acid (or its corresponding oxidation products, see below) and N-(hydroxymethyl)-3,4,5,6-tetrahydrophthalimide (MTI). MTI decomposed, presumably non-enzymatically (Suzuki and Miyamoto, 1974), into 3,4,5,6-tetrahydrophthalimide (TPI) which was subject to further extensive metabolic reactions, including reduction of the 1,2-double bond, hydroxylation at position 2 and 3, and full or partial hydrolysis of the carboxydimide moiety. Chrysanthemic acid was metabolised primarily by oxidation of the isobutenyl group. Subsequent conjugation reactions were reported for various phase I metabolites. *In vitro* studies using rat liver microsomes further demonstrated that oxidation reactions were NADPH-dependent, whereas a paraoxon-sensitive enzyme apparently mediated ester hydrolysis.

As a result of the extensive metabolism of tetramethrin, a large percentage of the resulting products (~50 %) remained unknown, unidentified or not extracted. Major labelled species identified at > 5 % in urine or faeces include the parent compound (5-30 % in faeces only), 3-hydroxy-cyclohexane-dicarboximide, 1-sulfo-cyclohexane-dicarboximide, N-(hydroxymethyl)-3-hydroxy-1-sulfo-cyclo-hexane-dicarboximide and three unknown metabolites. The sulfonic acid products were reported almost exclusively in faeces and result chemically from addition of sulfite to the 1,2-unsaturated bond of the tetrahydrophthalimide moiety.

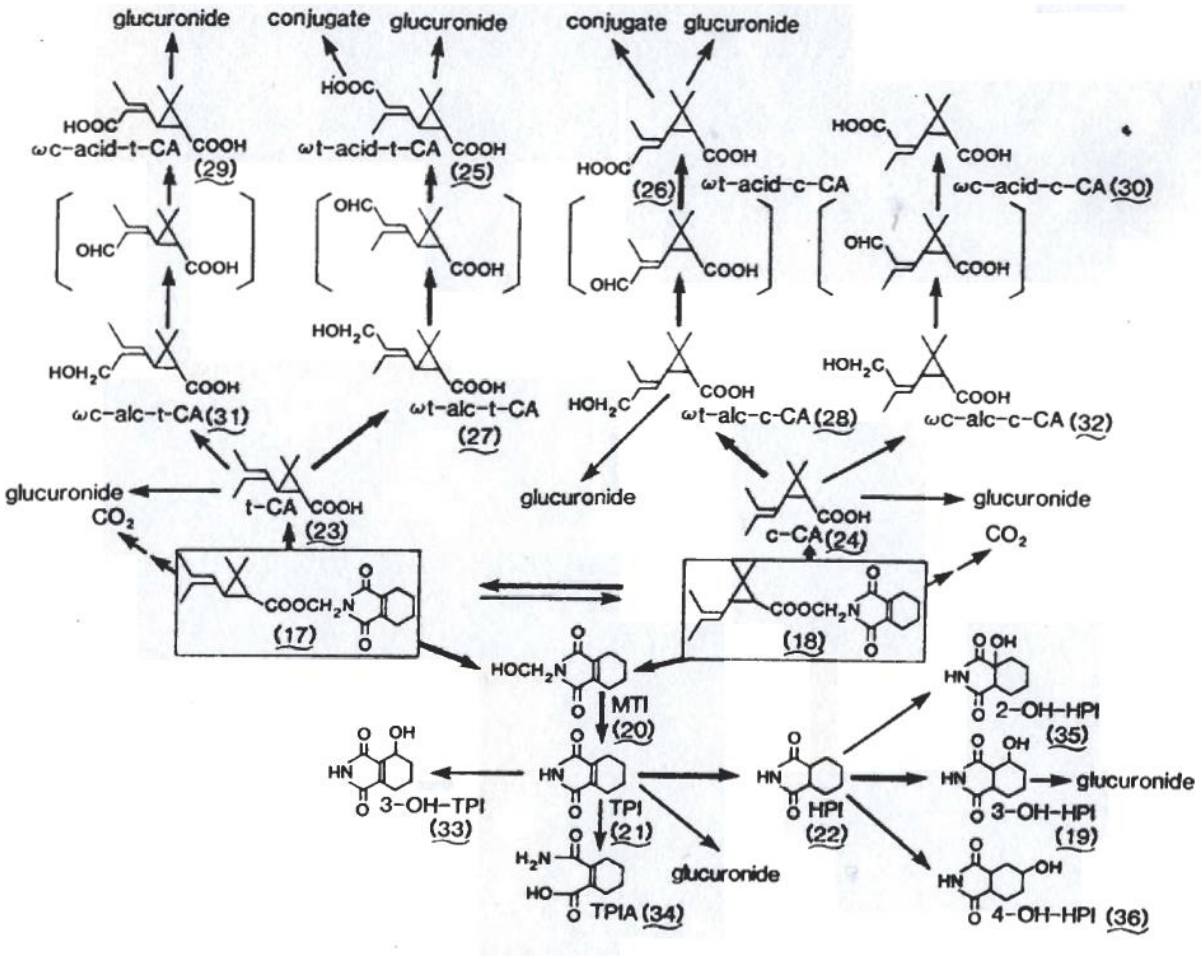


Figure 1: Metabolic pathways of tetramethrin in mammals (adopted from Environmental Health Criteria 98, World Health Organisation, Geneva, 1990).

In summary, the studies indicate sustained absorption of tetramethrin isomers from the G.I. tract which is estimated to be 50 % at doses in the range of the relevant oral NOAELs. Moreover, a very rapid systemic and/or pre-systemic metabolic degradation by oxidation and ester hydrolysis has been shown which is slower for cis- than for trans-isomers. Hence, the resulting levels of undegraded a.s. in blood and nervous system remain very low after oral administration (below 1 % of total C-14 at 1 h). However, bioavailability and systemic levels of parental compound may be different with other routes of application (inhalative, dermal). Excretion occurs via faeces and urine. Sulfonated metabolites in faeces are suggested to be a product of (intra)intestinal metabolism. Radiocarbon excretion is almost completed after 2-3 days. After 1 week C-14 tissue residues decreased to 0.4 % or below. There is no information that would justify usage of other assessment factors for inter- and intraspecies variation in toxicokinetics than the default values of 4 and 3.2, respectively. Mechanistically, there is potential for interference of organophosphates (paraoxon) with tetramethrin toxicokinetics.

Percutaneous absorption

Sumitomo provided a study on percutaneous absorption of technical products containing d-tetramethrin or tetramethrin. Substances were tested with 1 % solutions in ethanol on human heat-separated epidermis over 24 h, using a static diffusion cell set-up. Absorption into the receptor fluid (40 % acetone) was continuous over 24 h for d-tetramethrin but increased from 0.1 (0-8 h) to 0.3 (20-24 h) µg/cm^2/h for tetramethrin. As post-exposure separation of the upper stratum corneum from the remaining epidermis was not reliable due to deficiencies of the methodology (tissue samples disintegrated during tape stripping), both fractions were included in the calculation of overall skin absorption. For d-tetramethrin, a total of 12.4 ± 9.8 µg/cm^2, corresponding to 6.2 ± 4.9 % of the applied dose, was absorbed into and through the skin. 1.1 ± 1.3 and 3.5 ± 5.3 % of the doses were found in tissue layers attributed to stratum corneum and remaining epidermis, respectively. Totals of absorbed and absorbable dose were higher for tetramethrin, with 22.3 ± 5.8 µg/cm^2, corresponding to 11.2 ± 2.9 % of the applied dose, after 24 h. Apparent residues in the stratum corneum were higher than for d-tetramethrin with 13.8 µg/cm^2 (6.9 %), but lower in the remaining epidermis with 3.5 µg/cm^2 (1.8 %).

Applying the read-across concept established for d-tetramethrin and tetramethrin, and considering the limitations of the *in vitro* test system, an overall dermal absorption of 10 % is derived for tetramethrin and the technical product. Taking an estimated oral absorption of 50 % into account, the proposed value for dermal absorption (10 %) would also be supported by comparison of oral vs. dermal acute neurotoxicity of d-tetramethrin in the mouse (with adverse effects observed at 200-385 vs. 2500 mg/kg bw, see Evaluation of acute toxicity, Tables 25a and 26a).

Inhalative absorption

No experimental data are available for the derivation of internal doses achieved by absorption of inhaled tetramethrin or d-tetramethrin aerosol from the lung. Therefore, physiologically-based default assumptions must be used. As outlined in the corresponding Technical Guidance Document, respirable fractions of the test aerosol were calculated from measured aerodynamic particle sizes and, given the molecular weight of 331 g/mol and a logP of 4.3-4.6, 100 % of the respirable fraction was assumed to be the absorbable dose (European Commission, EUR 20418 EN/1, 2003). These assumptions are supported by experimental data obtained for other substances with similar physicochemical properties, e.g. prochlorperazine (MW 374 g/mol, LogP 4.6) with > 80 % inhalative absorption from a fine aerosol (Avram et al., 2007).

10. EVALUATION OF HEALTH HAZARDS

For assessment of human health hazards, read-across was performed between tetramethrin and d-tetramethrin.

Justification for read-across between tetramethrin and d-tetramethrin

Tetramethrins are esters of chrysanthemic acid with 3, 4, 5, 6-tetrahydrophthalimidomethyl alcohol and are classified as type I pyrethroids, which lack a cyano group within the alcohol moiety. Tetramethrin and d-tetramethrin are isomeric mixtures of [1R, cis], [1S, cis], [1R, trans] and [1S, trans]-tetramethrin, differing in the ratios of individual stereoisomers. The average isomeric composition of 5 batches of the technical products tetramethrin (Neo-Pynamin) and d-tetramethrin (Neo-Pynamin forte; both Sumitomo) as well as the range of isomeric composition derived from four

batches of tetramethrin (Duracide A; Endura) are shown in the table below. [1R, trans] and [1S, trans]-isomers prevailed in an analysis of the average batch composition of the corresponding technical product tetramethrin, while [1R, trans] is the main component (> 90 %) in d-tetramethrin.

Table 16: Average isomeric composition of technical products containing d-tetramethrin and tetramethrin (n=5; according to Fujita, Sumitomo Report No. SUP-0014, 2006; Endura: range derived from 4 batches)

Technical product	1R, cis (%)	1S, cis (%)	1R, trans (%)	1S, trans (%)
d-tetramethrin; Sumitomo	2.8	0.2	93.1	3.9
tetramethrin; Sumitomo	10.1	10.0	39.7	40.2
tetramethrin; Endura	9.1-10.3	7.6-10.3	40.0-46.9	35.5-40.4

The [1R, trans] isomer has been reported to be the isomer displaying the highest potential for activation of invertebrate axonal sodium channels (Lund and Narahashi, NeuroTox (1982) 3: 11-24).

All toxicological studies in mammals relate to isomeric mixtures. Thus, in the absence of comparative toxicological studies on the four isolated isomers, the toxicological potential of individual isomers in mammals has not been defined.

A review comparing the physico-chemical and biological properties of d-tetramethrin and tetramethrin was provided by the applicant Sumitomo (H. Roth, 2007/03/05, Safepharm Laboratories Ltd., Shardlow, UK). It was concluded that the two substances are essentially similar and “data on each of the compounds may be used to predict the toxicology and fate of the other”.

Indeed, a comparison of the submitted toxicological studies on d-tetramethrin and tetramethrin showed that, in cases in which both substances were investigated, the observed effects as well as NOAEL/LOAEL values were similar.

In rats, acute poisoning syndrome associated with type I pyrethroids typically is characterised by neurological effects such as aggressive sparring, whole body tremor and prostration (Verschoyle and Aldridge, 1980). Symptoms of neurotoxicity were observed for both tetramethrin and d-tetramethrin as a result of acute exposure and as acute effects during repeated inhalative exposure, including irregular respiration, bradypnoe and decreased spontaneous activity, whereas symptoms such as tremor, muscular fibrillation, urinary incontinence, hyperexcitability, ataxia, and limb paralysis were only observed in the acute inhalative toxicity study with d-tetramethrin.

After single oral administration, both tetramethrin and d-tetramethrin displayed only low acute toxicity in rats, with similar LOAELs for neurotoxicity (>2000 (Endura) 2500 mg/kg bw and 5000 mg/kg bw, resp.; both Sumitomo).

Regarding inhalative toxicity in rats, the LOAECs (converted to inhaled doses) for acute neurotoxic effects observed during exposure were within overlapping ranges for tetramethrin (13 mg/kg bw/d, 7 days, neonate mouse; 29.8 mg/kg bw/d, 90 d, rat) and d-tetramethrin (16.8 mg/kg bw, acute, rat; 11.2 mg/kg bw/d, 28 d, rat).

In repeated-dose rat studies, relevant adverse effects involved neurotoxic effects (propioceptive alterations), liver changes (weight increase, enlargement) supported by alterations of parameters in haematology and clinical chemistry (e.g. cholesterol, liver enzymes), although acute neurotoxic effects were also observed during daily exposure in inhalation toxicity studies. Only few studies with d-tetramethrin are available on subchronic/chronic toxicity (one 3/6 month rat oral toxicity study and

a 2-generation reproduction toxicity study). Notably, no carcinogenicity study has been provided for d-tetramethrin. However, on the basis of overlapping NOAEL-LOAEL intervals for tetramethrin and d-tetramethrin in medium-term (subacute/subchronic) and long-term studies, it can be concluded that tetramethrin and d-tetramethrin are of similar toxicological potency regarding liver changes and haematological findings.

For both tetramethrin and d-tetramethrin, no evidence for toxic effects on foetuses below doses causing maternal toxicity was provided in developmental toxicity studies.

Finally, neither tetramethrin nor d-tetramethrin met the criteria for classification as skin or eye irritating or as genotoxic. Both substances did not lead to sensitisation in guinea pig (Buehler) tests.

In summary, the comparison of the available data appears to scientifically justify risk assessment involving i) read-across of acute toxicity data, ii) unidirectional read-across from tetramethrin to d-tetramethrin for chronic toxicity, and iii) derivation of mutual NOAEL/LOAEL for all other types of toxicity.

Acute toxicity

10.1 ACUTE TOXICITY - ORAL ROUTE

Table 17: Summary table of animal studies on acute oral toxicity

Method, guideline, deviation(s) if any	Species, strain, sex, no/group	Test substance, reference to table 5	Dose levels, duration of exposure	Value LD_{50}	Reference
OECD 423 Oral, gavage	Rat, Wistar 3 F+3 M	Tetramethrin Vehicle: corn oil	2000 mg/kg bw	LD_{50}: > 2000 mg/kg bw No deaths and no toxic signs observed	Venugopala R K, 2002. Rallis Ltd, Endura Study No. 3335/01
Sim. to OECD 420 Oral, gavage	Rat, Sprague-Dawley, 5 M + 5 F	Tetramethrin Vehicle: corn oil	0-2500-5000 mg/kg bw	LD_{50}: > 5000 mg/kg bw No deaths ≥ 2500 mg/kg bw*:* Decrease in spontaneous activity, urinary incontinence, excretion of oily substance	Kawasaki H, 1990, Sumitomo Report No. IT-00-0224
Sim. to OECD 420 Oral, gavage	Rat, Crj:CD (SD), 5 M + 5 F	d-Tetramethrin Vehicle: corn oil	0-2500-5000 mg/kg bw	LD_{50}: > 5000 mg/kg bw; 5000 mg/kg bw: tremor, urinary incontinence, ataxic gait;	Misaki Y, 1999, Sumitomo Report No. SVT-0008

Method, guideline, deviation(s) if any	Species, strain, sex, no/group	Test substance, reference to table 5	Dose levels, duration of exposure	Value LD_{50}	Reference
				1 single death in the high dose group	
Pre-guideline, sim. to OECD 401, non-GLP Oral, gavage	Mouse, ddY, 10 M + 10 F	d-Tetramethrin Vehicle: corn oil	0-100-150-200-285-385-500-650-845-1000-1300-1700 mg/kg bw	LD_{50}: 1060/1040 mg/kg bw (M/F); 200 + 285 mg/kg bw/d: Slight decrease in spontaneous activity; ≥ 385 mg/kg bw: Hyperexcitaion, muscular fibrillation, ataxic gait, irregular respiration; ≥ 845 mg/kg bw: Whole body ataxia, weak respiration, salivation, mortalities	Kohda H, Misaki Y, Suzuki T, 1980, Sumitomo Report No. IT-00-0086

Table 18: Summary table of human data on acute oral toxicity

Type of data/report	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
No data		No cases of poisoning of humans with d-tetramethrin or tetramethrin have been reported		

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

In rats, tetramethrin displayed very low acute toxicity after oral administration, with no deaths at the limit dose of 2000 mg/kg bw (LD_{50} > 2000 mg/kg bw, Venugopala, 2002; Endura). This study was performed in accordance with OECD guideline 423 and confirmed the results of earlier studies in which d-tetramethrin and tetramethrin were tested up to oral doses of 5000 mg/kg bw and no mortality was observed (LD_{50} > 5000 mg/kg bw, Misaki, 1999; Kawasaki, 1990; both Sumitomo). Toxic effects were reversible within 3 days and comprised neurological symptoms (tremor, decrease in spontaneous activity, urinary incontinence) and excretion of oily substance.

In mice, the LD_{50} value for d-tetramethrin was 1040 mg/kg bw after oral administration and signs of toxicity were seen at 200 mg/kg bw and above. No cases of poisoning of humans with d-tetramethrin or tetramethrin have been reported.

10.1.2 Comparison with the CLP criteria

Table 19: Results of acute oral toxicity studies in comparison to the CLP criteria

Toxicological results	CLP criteria
Oral LD_{50} rat: > 2000 mg/kg bw (tetramethrin, d-tetramethrin) Oral LD_{50} mouse: 1040 mg/kg bw (F) (d-tetramethrin)	Cat 4 (H302): $300 < LD_{50} \leq 2000$ mg/kg bw (acute oral) Cat 3 (H301): $50 < LD_{50} \leq 300$ mg/kg bw (acute oral) Cat 2 (H300): $5 < LD_{50} \leq 50$ mg/kg bw (acute oral) Cat. 1 (H300): $LD_{50} \leq 5$ mg/kg bw (acute oral)

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Based on the available acute oral rat studies, tetramethrin / d-tetramethrin do not meet the criteria according to the CLP regulation for classification for acute oral toxicity. Although the oral LD_{50} value for mice was in the range for classification as ”Acute Tox. 4”, no classification for acute oral toxicity is proposed, taking into account that the single mouse study is a pre-guideline study and that results in mice are usually considered less relevant for classification. However, the final decision is within the remit of RAC/ECHA.

10.2 ACUTE TOXICITY - DERMAL ROUTE

Table 20: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviation(s) if any	Species, strain, sex, no/group	Test substance, reference to table 5	Dose levels duration of exposure	Value LD_{50}	Reference
OECD 402, dermal	Rat, Wistar 5 M + 5 F	Tetramethrin Vehicle: corn oil	2000 mg/kg bw; 24 h	LD_{50}: > 2000 mg/kg bw No toxic signs observed	Venugopala RK, 2002, Rallis Ltd., Endura Study No.° 3336/01, 14
Pre-guideline, sim. to OECD 402, non-GLP, dermal	Rat, Sprague-Dawley, 10 M + 10 F	d-Tetramethrin Vehicle: corn oil	0-2500-5000 mg/kg bw; 24 h	LD_{50}: > 5000 mg/kg bw No deaths and no toxic signs observed	Kohda H, Misaki Y, Suzuki T, 1980, Sumitomo Report No. IT-00-0086
Pre-guideline, sim. to OECD 402, non-GLP, dermal, occlusive	Rabbit, New Zealand White, 5 M + 5 F	Tetramethrin Vehicle: corn oil	0-2000 mg/kg bw; 24 h	LD_{50}: > 2000 mg/kg bw No deaths and no toxic signs observed	Suzuki T et al., 1987, Sumitomo Report No. IT-70-0207
Pre-guideline, sim. to OECD 402, non-GLP, dermal	Mouse, ddY, 10 M + 10 F	d-Tetramethrin Vehicle: corn oil	0-1000-2500-5000 mg/kg bw; 24 h	LD_{50}: > 5000 mg/kg bw; ≥ 2500 mg/kg bw: muscular fibrillation, irregular respiration	Kohda H, Misaki Y, Suzuki T, 1980, Sumitomo Report No. IT-00-0086

Table 21: Summary table of human data on acute dermal toxicity

Type of data/report	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
No data		No cases of poisoning of humans with d-tetramethrin or tetramethrin have been reported		

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

Dermal exposure of rats or rabbits to tetramethrin yielded no toxic effects at the limit dose of 2000 mg/kg bw (LD_{50} > 2000 mg/kg bw, Venugopala, 2002; Suzuki et al. 1987). Dermal exposure of rats to d-tetramethrin yielded no relevant toxic effects up to 5000 mg/kg bw in rats (Kohda et al., 1980). By contrast, mice displayed toxicity after dermal exposure to 2500 mg/kg bw d-tetramethrin (muscular fibrillation, irregular respiration), but the LD_{50} was estimated to be above 5000 mg/kg bw (Kohda et al. 1980).

10.2.2 Comparison with the CLP criteria

Table 22: Results of acute dermal toxicity studies in comparison to the CLP criteria

Toxicological results	CLP criteria
Dermal LD_{50} rat: > 2000 mg/kg bw (tetramethrin) Dermal LD_{50} rat: > 5000 mg/kg bw (d-tetramethrin) Dermal LD_{50} rabbit: > 2000 mg/kg bw (tetramethrin) Dermal LD_{50} mouse: > 5000 mg/kg bw (d-tetramethrin)	Cat 4 (H312): $1000 < LD_{50} \leq 2000$ mg/kg bw (acute dermal) Cat 3 (H311): $200 < LD_{50} \leq 1000$ mg/kg bw (acute dermal) Cat 2 (H310): $50 < LD_{50} \leq 200$ mg/kg bw (acute dermal) Cat. 1 (H310): $LD_{50} \leq 50$ mg/kg bw (acute dermal)

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Based on the submitted acute dermal toxicity studies, tetramethrin and d-tetramethrin do not meet the criteria for classification for acute dermal toxicity.

10.3 ACUTE TOXICITY - INHALATION ROUTE

Table 23: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviation(s) if any	Species, strain, sex, no/group	Test substance, reference to table 5, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
OECD 403 Acute inhalation toxicity study with Tetramethrin in Wistar rats. Head and nose exposure Technical deficiencies: Volume of air chamber 500 L, suggesting long time to achieve steady-state of concentration; no indication of whether concentration	Rat, Wistar Pre-study (G1): 2 M + 2 F Main study (G2): 5 M + 5 F	Tetramethrin aerosol in cyclohexanone (50% w/v); mean aerosol particle size: G1: 0.67 ± 0.26 µm; G2: 0.68 ± 0.26 µm	0-5.63 ± 0.86 mg/L; 4 h	LC_{50}: > 5.63 mg/L Slight lacrimation and nasal discharge on day 1 (all rats in G2), normal from day 2 onwards), otherwise no toxic signs observed Absence of signs of acute neurotoxicity in comparison to other studies suggests that concentration of 5.63 mg/L may not have been	Venugopala RK, 2006, Toxicology Department, Advinus Therapeutics Private Limited., Endura Study N° 4414/05

Method, guideline, deviation(s) if any	Species, strain, sex, no/group	Test substance, reference to table 5, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
measurements performed in breathing zone; dense aerosol accumulation in chamber of G2 groups				achieved in the breathing zone, study not appropriate for classification purposes	
Pre-guideline, non-GLP. Acute inhalation, whole body 3 h exposure instead of 4 h; Dose-finding study for a subacute (28 day) study	Rat, Sprague-Dawley, 10 M + 10 F	d-Tetramethrin dissolved in: deodorised kerosene mist, particle size 1.23-1.51 µm	0-0.026-0.131-0.243-0.595-1.18 mg/L (approx. 0-3.3-16.8-32.3-76.3-151 mg/kg bw); 3 h	LC_{50}: > 1.18 mg/L (151 mg/kg bw); ≥ 0.131 mg/L: Muscular fibrillation, urinary incontinence, limb paralysis, bradypnoe, irregular respiration respiration (no. of affected animals and severity of findings not reported; toxic signs began to appear 15 to 30 min, after initiation of exposure and disappeared 1 to 2 hours after exposure) at 1.18 mg/L: 1/10 females died	Suzuki T, Kohda H, Misaki Y, Okuno Y, Koyama Y, Miyamoto J, 1981, Sumitomo Report No. IT-10-0144
28 day inhalation, whole body 3 h / day exposure	Rat, Sprague-Dawley 10 M + 10 F	d-Tetra-methrin dissolved in: deodorised kerosene mist, particle size 1.23-1.51 µm	0-0.026-0.049-0.087 mg/L 3 h / day exposure for 7 days aweek	≥ 0.087 mg/L: Slight bradypnea, irregular respiration, salivation directly after exposure (no. of affected animals and severity of findings not reported), no cumulative effect	Suzuki T, Kohda H, Misaki Y, Okuno Y, Koyama Y, Miyamoto J, 1981, Sumitomo Report No. IT-10-0144

Method, guideline, deviation(s) if any	Species, strain, sex, no/group	Test substance, reference to table 5, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
				Increase in leucocyte and decrease in eosinophils count at 0.087 mg/l NOAEL: 0.049 mg/L	

Table 24: Summary table of human data on acute inhalation toxicity

Type of data/report	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
No data		No cases of poisoning of humans with d-tetramethrin or tetramethrin have been reported.		

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

An acute rat inhalation toxicity study with d-tetramethrin and exposure time of 3 hours revealed moderate toxicity, with systemic effects occurring in the groups at 0.131 mg/L (corresponding to approx. 16.8 mg/kg bw) and above: decreases of spontaneous activity, salivation, hyperexcitability, hyperpnoea, irregular respiration, urinary incontinence, muscular fibrillation, ataxia, limb paralysis and other toxic signs were observed (Suzuki et al. 1981) (no. of affected animals and severity of findings not reported). The NOAEC of 0.026 mg/L was estimated to correspond to 3.3 mg/kg bw, the conversion of inhaled concentrations into inhaled doses being based on default assumptions regarding body weight, inhalation volume and 100 % availability. The LC50 could only be estimated as being > 1.18 mg/L (> 151 mg/kg bw), with 1/20 mortality at this concentration. The RMS notes that the duration of exposure in the study was shorter than recommended according to OECD 403 (only 3 hours instead of 4) and that more severe effects might be anticipated for the standard exposure of 4 hours. Higher concentrations for refining the LC50 were not tested in this study by Suzuki et al. 1981, but appear technically feasible, since an almost linear relationship was recognised between substance concentration injection and aerial concentration. By contrast, the results of the study by Suzuki et al. 1981 were not confirmed in an acute rat inhalation toxicity study using tetramethrin and an exposure time of 4 hours in accordance with OECD guideline 403. No mortality was observed up to the highest concentration tested. Hence, the LC50 was estimated to be > 5.63 mg/L. Slight lacrimation and nasal discharge occurred on day 1, which was not observed from day 2 post exposure onwards. No other clinical signs were observed (Venugopala 2006; Endura). Due to dense aerosol accumulation inside the chamber, observation of the animals during exposure was not possible. Thus, potential clinical signs that may occur only during but not after exposure could not have been recorded. In addition, it is not clear which aerosol concentrations were actually achieved in the breathing zone. In contrast to other inhalation studies performed with d-tetramethrin (the acute rat study by Suzuki et al. 1981 and

the 28-day study by Suzuki et al. 1981) or tetramethrin (90-day study performed by Kawaguchi 1991, see Table 38d), no clinical signs of acute neurotoxicity were reported in the study by Venugopala. In conclusion, the acute rat inhalation study by Venugopala is regarded by the RMS as being less relevant to considerations concerning classification and labelling, due to deficiencies in conduction of the study and the total absence of expected acute neurotoxicity.

10.3.2 Comparison with the CLP criteria

Table 25: Results of acute inhalation toxicity studies in comparison to the CLP criteria

Toxicological results	CLP criteria
Inhalative LD_{50} rat: > 1.18 mg/L, (d-tetramethrin mist; 1/20 mortality at 1.18 mg/L, exposure for only 3 h)	Cat 4 (H332): $10 < LD_{50} \leq 20$ mg/L (acute inhalation, vapours) $1 < LD_{50} \leq 5$ mg/L (dusts and mists) Cat 3 (H331): $2.0 < LD_{50} \leq 10$ mg/L (vapours) $0.5 < LD_{50} \leq 1$ mg/L (dusts and mists) Cat 2 (H330): $0.5 < LD_{50} \leq 2$ mg/L (vapours) $0.05 < LD_{50} \leq 0.5$ mg/L (dusts and mists) Cat. 1 (H330): $LD_{50} \leq 0.5$ mg/L (vapours) $LD_{50} \leq 0.05$ mg/L (dusts and mists) Exposure generally for 4 h

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

From the data presented in the study by Suzuki et al. 1981 for d-tetramethrin, indicating an LD50 above 1.18 mg/L (observed mortality at 1.18 mg/L), and considering that rats were only exposed for 3 h, it cannot be ruled out that the LC50 is ≤ 5 mg/L. Thus, classification of d-tetramethrin and tetramethrin as "Acute Tox. 4; H332" is proposed.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification of d-trans-tetramethrin for acute oral toxicity on the basis of the following data:

- One OECD test guideline (TG) 423 study in rat showing no mortalities at 2000 mg tetramethrin/kg bw;
- One study similar to OECD TG 420 in rat showing no mortalities at 5000 mg tetramethrin/kg bw;

- One study similar to OECD TG 420 in rat showing one mortality among 10 animals at 5000 mg d-trans-tetramethrin/kg bw;
- One non-GLP study similar to OECD TG 401 in mouse showing an LD_{50} of 1060/1040 mg/kg bw (males/females) for d-trans-tetramethrin.

The DS proposed no classification of d-trans-tetramethrin for acute dermal toxicity on the basis of the following data:

- One OECD TG 402 study in rat showing an LD_{50} above 2000 mg tetramethrin/kg bw;
- One study similar to OECD TG 402 in rabbit showing an LD_{50} above 2000 mg tetramethrin/kg bw;
- One non-GLP study similar to OECD TG 402 in rat showing an LD_{50} above 5000 mg d-trans-tetramethrin/kg bw;
- One non-GLP study similar to OECD TG 402 in mouse showing an LD_{50} above 5000 mg d-trans-tetramethrin/kg bw.

The DS proposed classification of d-trans-tetramethrin for acute inhalation toxicity as Category 4 (H332) on the basis of a pre-guideline non-GLP study (designed as a dose-finding study for a sub-acute 28-day toxicity study) with d-trans-tetramethrin. In this study, Sprague-Dawley rats were exposed during 3 hours to 5 different concentrations (whole body exposure) of d-trans-tetramethrin. The study resulted in 1 female and 0 male mortalities at 1.18 mg/mL among a total of 10 animals of each sex. The DS postulated the possibility that the LC_{50} after 4 hours of exposure can be lower than 5 mg/L.

The DS considered another tetramethrin OECD TG 403 study performed in rats, showing an LC_{50} above 5.63 mg/L, as not reliable for classification purposes due to technical deficiencies. Specifically: i) the volume of the air chamber was 500 L suggesting it would take a long time to achieve the steady-state concentration; ii) there were no indications of whether concentrations measurements were performed in the breathing zone; and, iii) a dense aerosol accumulation in the chamber was described.

Comments received during public consultation

One MSCA supported the classification proposal.

One MSCA expressed that the exact determination of the LC_{50} for tetramethrin is not possible with only one death in 20 animals and therefore the DS's assumption about the possibility that the LC_{50} might be lower than 5 mg/L, thus supporting classification as Category 4, is not sufficiently justified, especially considering the gap of information regarding the cause and time of death. The DS replied that the signs of neurotoxicity began 15-30 minutes after the initiation of the exposure and disappeared 1-2 hours after the end of it. However, the incidence of the neurotoxicity and time of the death was not reported. The DS also clarified that the neurotoxic signs were also noted after each single exposure and in a non-accumulative way in the sub-chronic inhalation toxicity study. All these considerations and the well-known neurotoxic mechanism of action of pyrethroids allowed the DS to propose that the lethality was due to neurotoxicity.

Additional key elements

The three tables below summarise the acute oral, dermal and inhalation toxicity studies, respectively, that were reported by the DS in the CLH report for tetramethrin. No cases of poisoning with humans with tetramethrin have been reported.

Table: Summary of acute oral toxicity studies with tetramethrin. In all cases the vehicle corn oil.

Method	Species Sex Nº group	Dose level	Results	Reference
OECD TG 423 Oral, gavage	Rat Wistar 3 F + 3 M	2000 mg/kg bw	LD_{50} > 2000 mg/kg bw No deaths and no toxic signs observed	Venugopala, 2002
Similar to OECD TG 420 Oral, gavage	Rat Sprague-Dawley 5 M + 5 F	0-2500-5000 mg/kg bw	LD_{50} > 5000 mg/kg bw No deaths ≥ 2500 mg/kg bw: Decrease in spontaneous activity, urinary incontinence, excretion of oily substance	Kawasaki, 1990

Table: Summary of acute dermal toxicity studies with tetramethrin. In all cases the vehicle corn oil.

Method	Species Sex Nº group	Dose level	Results	Reference
OECD TG 402	Rat Wistar 5 M + 5 F	2000 mg/kg bw 24 h	LD_{50} > 2000 mg/kg bw No toxic signs observed	Venugopala, 2002
Pre-guideline Similar to OECD TG 402 Non-GLP Occlusive	Rabbit New Zealand White 5 M + 5 F	0-2000 mg/kg bw 24 h	LD_{50} > 2000 mg/kg bw No deaths and no toxic signs observed	Suzuki *et al.*, 1987

Table: Summary of acute inhalation toxicity study with tetramethrin.

Method	Species Sex Nº group	Dose level	Results	Reference
OECD TG 403	Rat	5.63 ± 0.86 mg/L	LC_{50} > 5.63 mg/L	Venugopala, 2006

Head and nose exposure	Wistar Preliminary study (G1): 2 M + 2 F Main study (G2): 5 M + 5 F	 4 hours Aerosol in cyclohexanone (50% w/v); Mean aerosol particle size: G1: 0.67 ± 0.26 µm G2: 0.68 ± 0.26 µm	Slight lacrimation and nasal discharge on day 1 (all rats in G2), normal from day 2 onwards, No toxic signs observed	

Assessment and comparison with the classification criteria

The three tables below summarise the acute oral, dermal and inhalation animal toxicity studies, respectively, that were reported by the DS in the CLH report for d-trans-tetramethrin. No cases of poisoning with humans with d-trans-tetramethrin have been reported.

Table: Summary of acute oral toxicity studies with d-trans-tetramethrin. In all cases the vehicle corn oil.

Method	Species Sex Nº group	Dose level	Results	Reference
Similar to OECD TG 420 Oral (gavage)	Rat Crj:CD (SD) 5 M + 5 F	0, 2500, 5000 mg/kg bw	LD_{50} > 5000 mg/kg bw 5000 mg/kg bw: Tremor, urinary incontinence, ataxic gait (reversible within 4 days) 1 single death in the high dose group	Misaki, 1999
Pre-guideline Similar to OECD TG 401 Non-GLP Oral (gavage)	Mouse ddY 10 M + 10 F	0, 100, 150, 200, 285, 385, 500, 650, 845, 1000, 1300, 1700 mg/kg bw	LD_{50} = 1060/1040 mg/kg bw (M/F) 200 & 285 mg/kg bw: Slight decrease in spontaneous activity ≥ 385 mg/kg bw: Hyperexcitaion, muscular fibrillation, ataxic gait, irregular respiration ≥ 845 mg/kg bw: Whole body ataxia, weak respiration, salivation, mortalities	Kohda, Misaki, Suzuki, 1980

Table: Summary of acute dermal toxicity studies with d-trans-tetramethrin. In all cases the vehicle corn oil.

Method	Species Sex Nº group	Dose level	Results	Reference
Pre-guideline Similar to OECD TG 402 Non-GLP	Rat Sprague-Dawley 10 M + 10 F	0, 2500, 5000 mg/kg bw 24 h	LD_{50} > 5000 mg/kg bw No deaths and no toxic signs observed	Kohda *et al*., 1980
Pre-guideline Similar to OECD TG 402 Non-GLP	Mouse ddY 10 M + 10 F	0, 1000, 2500, 5000 mg/kg bw 24 h	LD_{50} > 5000 mg/kg bw; ≥ 2500 mg/kg bw: Muscular fibrillation, irregular respiration	Kohda *et al*., 1980

Table: Summary of acute inhalation toxicity study with d-trans-tetramethrin.

Method	Species Sex Nº group	Dose level	Results	Reference
Pre-guideline Non-GLP Whole body 3 h exposure Dose-finding study for a subacute (28-day) study	Rats Sprague-Dawley 10 M + 10 F	0, 0.026, 0.131, 0.243, 0.595, 1.18 mg/L Dissolved in deodorised kerosene Mist particle size =1.23-1.51 µm	LC_{50} > 1.18 mg/L ≥ 0.131 mg/L: Muscular fibrillation, urinary incontinence, limb paralysis, bradypnoe, irregular respiration (number of affected animals and severity of findings not reported; toxic signs began to appear 15 to 30 min after initiation of exposure and disappeared 1 to 2 hours after exposure) 1.18 mg/L: 1/10 females died	Suzuki *et al*., 1981

Comparison with the criteria

The overall analysis of the available information for acute oral toxicity of tetramethrin shows two studies with rat causing no mortalities at doses of 2000 and 5000 mg/kg bw. One oral study in rat with d-trans-tetramethrin caused a single mortality at 5000 mg/kg bw. Finally, a study in mouse with d-trans-tetramethrin showed neurotoxicity and LD_{50} of 1050 mg/kg bw (combined for both sex). It suggest that mice might be more sensitive to tetramethrins than rat and, according to the "Guidance on the Application of the CLP Criteria" the most sensitive species should be used for setting classification. Thus, the LD_{50} in the most sensitive species is higher than 300 and lower than 2000 mg/kg bw/day and consequently RAC concluded that d-trans-tetramethrin be classified as **Acute Toxicity Category 4, via oral route (H302: Harmful if swallowed).**

The limit concentration for triggering classification for acute dermal toxicity is 2000 mg/kg bw. The available information shows that doses of up to 5000 mg/kg bw tetramethrin and d-trans-tetramethrin did not cause mortalities. Thus, RAC agreed with the DS that d-trans-tetramethrin **does not fulfill the criteria for classification for acute dermal toxicity.**

The DS presented an acute rat inhalation toxicity study with d-trans-tetramethrin and exposure time of 3 hours revealing moderate toxicity, with toxic systemic effects (decreases of spontaneous activity, salivation, hyperexcitability, hyperpnea, irregular respiration, urinary incontinence, muscular fibrillation, ataxia, limb paralysis etc.) occurring in the groups exposed to 0.131 mg/L and above. In this study, at the highest dose (1.18 mg d-trans-tetramethrin/L) only 1 female among 10 died, while no mortalities were reported among the 10 exposed males. Based on this, the DS postulated that the LC_{50} for 4 hours of exposure might be lower than 5 mg/L and proposed classification as Acute Toxicity Category 4. RAC is however of the opinion that, with only 5% of mortalities (1 female among 10 females and 10 males) reported at 1.18 mg d-trans-tetramthrin/L, it is not possible to establish if the LD_{50} could be higher or lower than 5 mg/L. Therefore, based on the available evidence RAC concluded on **no classification** of d-trans-tetramethrin for **acute inhalation toxicity.**

10.4 SKIN CORROSION/IRRITATION

Table 26: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
OECD 404	Rabbit, New Zealand White, 3 M	Tetramethrin, as paste with corn oil	500 mg, 4 h	1, 24, 48, 72 h: No erythema, oedema or any other effects on skin observed; Reversibility: N/A Not irritating	Mohan Kumar, 2002, Rallis Ltd., Endura Study N° 3337/01
OECD 404	Rabbit, New Zealand White, 3 M + 3 F	Tetramethrin, Moistened with corn oil	500 mg, 4h	0.5, 24, 48, 72 h: No erythema, oedema or any other effects on skin observed; Reversibililty: N/A Not irritating	Nakanishi T, 1990, Sumitomo Report No. IT-00-0217
Pre-guideline, sim. to OECD 404, non-GLP	Rabbit, Albino, 6 M	d-Tetramethrin	0.5 ml of formulation 24 h, Intact and abraded skin, occlusive	24, 48, 72 h and 1 week: No erythema, oedema or any other effects on skin observed; Reversibility: N/A Not irritating	Hara S, Suzuki T, 1980, Sumitomo Report No. IT-00-0073

Table 27: Summary table of human data on skin corrosion/irritation

Type of data/report	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
No data				

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

Studies on skin irritation revealed that neither d-tetramethrin nor tetramethrin is irritating to the skin of rabbits.

10.4.2 Comparison with the CLP criteria

Table 28: Results of skin irritation studies in comparison to the CLP criteria

Toxicological results	CLP criteria
No erythema, oedema or any other skin effects observed	Irritating to skin (Category 2, H315): at least in 2/3 tested animal a positive response of: Mean value of ≥ 2.3 - ≤ 4.0 for erythema/eschar or for oedema

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

No classification/labelling for skin irritation is proposed for tetramethrin or d-tetramethrin

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification of d-trans-tetramethrin for skin irritation/corrosion on the basis of the following data:

- Two independent OECD TG 404 tests with New Zealand White (NZW) rabbits showed that 500 mg (4 hours of exposure) of tetramethrin were not able to induce erythema, oedema or any other effects on skin up to 72 hours after the exposure.
- One non-GLP pre-guideline study similar to OECD TG 404 with Albino rabbits showed that 24 hours of exposure to 0.5 mL of d-trans-tetramethrin formulation were unable to induce erythema, oedema or any other effects on skin up to 1 week after exposure.

Comments received during public consultation

One MSCA supported the no classification proposal.

Additional key elements

The table below summarises the available skin corrosion/irritation study with tetramethrin.

Table: Summary of animal studies on skin corrosion/irritation with tetramethrin.

Method	Species Strain Sex N°/group	Dose levels Duration of exposure	Results Observations and onset Mean scores/animal Reversibility	Reference
OECD TG 404	Rabbit NZW 3 M	500 mg as paste with corn oil 4 h	1, 24, 48, 72 h: No erythema, oedema or any other effects on skin observed Reversibility: N/A	Mohan Kumar, 2002
OECD TG 404	Rabbit NZW 3 M + 3 F	500 mg moistened with corn oil 4h	0.5, 24, 48, 72 h: No erythema, oedema or any other effects on skin observed Reversibililty: N/A	Nakanishi, 1990

Assessment and comparison with the classification criteria

The table below summarises the available skin corrosion/irritation studies with d-trans-tetramethrin.

Table: Summary of animal study on skin corrosion/irritation with d-trans-tetramethrin.

Method	Species Strain Sex N°/group	Dose levels Duration of exposure	Results Observations and onset Mean scores/animal Reversibility	Reference
Pre-guideline Similar to OECD TG 404 Non-GLP	Rabbit Albino 6 M	0.5 mL of formulation 24 h Intact and abraded skin Occlusive	1, 24, 48, 72, 96 h and 1 week: No erythema, oedema or any other effects on skin observed Reversibility: N/A	Hara and Suzuki, 1980

In conclusion, the available studies revealed that neither tetramethrin nor d-trans-tetramethrin is irritating to skin of rabbits and therefore, **RAC agreed with the DS that no classification** of d-trans-tetramethrin for **skin corrosion/irritation is warranted.**

10.5 SERIOUS EYE DAMAGE/EYE IRRITATION

Table 29: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
OECD 405	Rabbit, New Zealand white, 3 M	Tetramethrin, mixed in corn oil	53 mg (equivalent to 0.1 ml, test substance mixted in corn oil) 24 h	1, 24, 48, 72 h: No ocular lesions observed; Reversibility: N/A Not irritating	Mohan Kumar SB, 2002, Rallis Ltd., Endura Study N° 3338/01
OECD 405	Rabbit, New Zealand White, 3 M + 3 F	Tetramethrin	100 mg Exposure period: N/A	Observation times (times after application): 1, 24, 48, 72 h; Corneal opacity: 2/6 animals with grade 1 at 24 h; Iris: No signs of irritation observed; Conjunctival redness: 6/6 animals with grade 1 at 1 h; 3/6 animals with grade 1 at 24 h; Conjunctival chemosis: 5/6 animals, grade 1 at 1 h; Reversibility of all effects: Yes, within 48 h of application Not irritating (below classification threshold)	Nakanishi T, 1990, Sumitomo Report No. IT-00-0217
Pre-guideline, non GLP	Rabbit, Albino, 6 M (eyes not washed after application) 3M (eyes washed	d-Tetramethrin	0.1 ml instilled Exposure period: Eyes of 6 animals unwashed after application; Eyes of 3 animals washed 30 seconds after	Observation times at 1, 24, 48 h and at 1 week Cornea: No signs of irritation observed Iris: No signs of irritation observed Conjunctiva: slight hyperaemia and/or chemosis 1 h (grade 1) after application (unwashed group); slight hyperaemia (grade 1) 1 h after application in the washed group Reversibility: Yes, within 48 h	Hara S, Suzuki T, 1980, Sumitomo Report No. IT-00-0073

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance , reference to table 5	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
	applicatio n)		application	Not irritating (below classification threshold)	

Table 30: Summary table of human data on serious eye damage/eye irritation

Type of data/report	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
No data				

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

When applied to rabbit eyes, tetramethrin displayed no eye irritation (Mohan Kumar, 2002) or minimal eye irritating potential (Naganishi, 1990). Similarly, in a pre-guideline study, d-tetramethrin elicited only minimal signs of irritation (Hara and Suzuki, 1980). Any slight effects observed in the rabbit studies (on cornea and conjunctiva for tetramethrin, or on conjunctiva for d-tetramethrin) were reversible by 48 hours after application.

10.5.2 Comparison with the CLP criteria

Table 31: Results of eye irritation studies in comparison to the CLP criteria

Toxicological results	CLP criteria
Tetramethrin: Study 1: Not irritating (Mohan Kumar, 2002); Study 2: 2/6 animals positive for corneal opacity, grade 1, at 24 h and 6/6 animals positive for conjunctival redness and/or chemosis, grade 1, at 1 h (Nakanishi , 1990) d-Tetramethrin: Slight hyperaemia and/or chemosis, grade 1, 1 h after application	Irritating to eyes (Category 2, H319): at least in 2/3 tested animal a positive response of: corneal opacity: ≥ 1 and/or iritis: ≥ 1 and/or conjunctival redness: ≥ 2 and/or conjunctival oedema (chemosis): ≥ 2

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

Based on the available data, classification of tetramethrin and d-tetramethrin for serious eye damage/eye irritation is not proposed.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification of d-trans-tetramethrin for serious eye damage/irritation on the basis of the following information:

- One OECD study with NZW rabbits showing that 53 mg of tetramethrin (24 hours of exposure) caused no ocular lesion after 72 hours of observation;
- One OECD study with NZW rabbits showed that 100 mg of tetramethrin (time of exposure not known) caused reversible corneal opacity and conjunctival redness of grade ≤ 1;
- One non-GLP pre-guideline study with Albino rabbits showed that 0.1 mL of instilled d-trans-tetramethrin (total amount of product not known) caused reversible slight hyperaemia in conjunctiva of grade ≤ 1.

Comments received during public consultation

One MSCA supported the no classification proposal.

Additional key elements

The table below summarises the available eye corrosion/irritation study with tetramethrin.

Table: Summary of animal study on serious eye damage/irritation with tetramethrin.

Method	Species Strain Sex Nº/group	Dose levels Duration of exposure	Results Observations and onset Mean scores/animal Reversibility	Reference
OECD TG 405	Rabbit NZW 3 M	53 mg (equivalent to 0.1 mL, test substance mixted in corn oil) 24 h	1, 24, 48, 72 h: No ocular lesions observed Reversibility: N/A	Kumar, 2002
OECD TG 405	Rabbit NZW 3 M + 3 F	100 mg Exposure period: N/A	Observation (times after application): 1, 24, 48, 72 h <u>Corneal opacity:</u> 2/6 animals with grade 1 at 24 h <u>Iris:</u> No signs of irritation observed	Nakanishi, 1990

			Conjunctival redness: 6/6 animals with grade 1 at 1 h; 3/6 animals with grade 1 at 24 h Conjunctival chemosis: 5/6 animals, grade 1 at 1 h Reversibility of all effects: Yes, within 48 h of application	

Assessment and comparison with the classification criteria

Table below summarises the available eye corrosion/irritation study with d-trans-tetramethrin.

Table: Summary of animal study on serious eye damage/irritation with d-trans-tetramethrin.

Method	Species Strain Sex Nº/group	Dose levels Duration of exposure	Results Observations and onset Mean scores/animal Reversibility	Reference
Pre-guideline non GLP	Rabbit Albino 9 M	0.1 ml instilled Eyes of 6 animals unwashed after application Eyes of 3 animals washed 30 seconds after application	Observation times: 1, 24, 48 h and at 1 week Cornea: No signs of irritation observed Iris: No signs of irritation observed Conjunctiva: slight hyperaemia and/or chemosis 1 h (grade 1) after application (unwashed group); slight hyperaemia (grade 1) 1 h after application in the washed group Reversibility: Yes, within 48 h	Hara and Suzuki, 1980

The CLP Regulation states that for classification in the lowest category for eye damage at least 2/3 tested animal should display a positive response of: corneal opacity ≥ 1 and/or iritis ≥ 1 and/or conjunctival redness ≥ 2 and/or conjunctival oedema (chemosis) ≥ 2. The highest mean score at 24, 48 and 72 hours for corneal opacity was grade 1, but it appeared in 2 out of 6 animals and effects on at least 4 would be necessary for warranting classification. The

conjunctival redness and chemosis appeared with a highest mean score of 1 at 24, 48 and 72 hours, and a minimum grade of 2 is needed for warranting classification. No iris damages were noted in any study. Another independent study reported no ocular lesions after tetramethrin instillation. Thus, RAC concurred with the DS and concluded on **no classification** of d-trans-tetramethrin **for serious eye damage/irritation.**

10.6 RESPIRATORY SENSITISATION

Table 32: Summary table of animal studies on respiratory sensitisation

Method, guideline, deviations if any	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels, duration of exposure	Results	Reference
No studies available					

Table 33: Summary table of human data on respiratory sensitisation

Type of data/report	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
Factory workers' surveillance data	Tetramethrin	Regular medical examination, blood, hepatic, renal and urine analysis, spirometry, biological monitoring, audiometry, ergovision 65 workers	No findings attributable to exposure	Dr. Savron L, 2006, Endura S.p.A .- Ravenna Plant Medical Data. Safety, Environment and quality department, 11 April 2006
Factory worker examination review		Regular medical check-up (bw, visual and auditory acuity, chest x-ray, blood pressure, urinalysis, serum biochemistry), 7 workers exposed to pyrethroids incl. tetramethrin dermally and by inhalation during packaging	No findings attributable to exposure	Shono F, 2005, Sumitomo Report No. SVT-0009
Case report	Tetramethrin	Single case of professional (M) developing asthma after 6 years of work as exterminator	Skin prick testing to tetramethrin negative Challenge-provoked reactions: Reduced respiratory function (reduced forced	Vandenplas O, Delwiche J P, Auverdin J, Caroyer U M, Cangh F B,

Type of data/report	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
		Inhalation challenges: 1st challenge: formulation containing tetramethrin + organophosphate; 2nd challenge (5 mo. after first): Tetramethrin powder diluted 1/10 in lactose powder	expiratory volume in 1 second; asthma) Patient treated with beta-agonist when required	2000, Allergy 55(4): 417-418

10.6.1 Short summary and overall relevance of the provided information on respiratory sensitisation

Medical surveillance of workers exposed to pyrethroids, including d-tetramethrin and tetramethrin, did not provide evidence for significant adverse effects. A single case study (Vandenplas et al., 2000) indicates that individual asthmatic reactions against tetramethrin may to be possible. However, with regard to tetramethrin, epidemiological studies on exposed populations are not available. No animal studies involving respiratory sensitisation are available.

10.6.2 Comparison with the CLP criteria

Table 34: Information on respiratory sensitisation in comparison to the CLP criteria

Toxicological results	CLP criteria
One case study on single individual, showing reaction after challenge to tetramethrin-containing formulation or tetramethrin powder (reduced respiratory function, asthma)	Cat 1: Based on evidence in humans that the substance can lead to specific respiratory hypersensitivity and/or Positive results from appropriate animal test

10.6.3 Conclusion on classification and labelling for respiratory sensitisation

Due to paucity of data, no classification of tetramethrin or d-tetramethrin for respiratory sensitisation is proposed.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

The DS proposed no classification for respiratory sensitisation because after the analysis of factory worker's surveillance data in two different studies, one with 65 workers and a second one with 7 workers, only a single case study showed individual asthmatic reactions due to tetramethrin.

Comments received during public consultation

One MSCA supported the no classification proposal.

Assessment and comparison with the classification criteria

The table below summarises the factory workers' surveillance data available on respiratory sensitization.

Table: Summary table of human data on respiratory sensitisation

Type of data/Report	Test substance	Relevant information	Observations	Reference
Factory workers' surveillance data	Tetramethrin	Regular medical examination, blood, hepatic, renal and urine analysis, spirometry, biological monitoring, audiometry, ergovision 65 workers	No findings attributable to exposure	Savron, 2006
Factory worker examination review		Regular medical check-up (bw, visual and auditory acuity, chest x-ray, blood pressure, urinalysis, serum biochemistry) 7 workers exposed to pyrethroids, including tetramethrin dermally and by inhalation during packaging	No findings attributable to exposure	Shono 2005
Case report	Tetramethrin	Single case of professional (M) developing asthma after 6 years of work as exterminator <u>Inhalation challenges:</u> 1st challenge: formulation containing tetramethrin + organophosphate; 2nd challenge (5 months after first):	Skin prick testing to tetramethrin negative Challenge-provoked reactions: Reduced respiratory function (reduced forced expiratory	Vandenplas *et al*., 2000

		Tetramethrin powder diluted 1/10 in lactose powder	volume in 1 second; asthma) Patient treated with beta-agonist when required	

The CLP Regulation establishes that substances shall be classified as respiratory sensitisers (Category 1) if there is evidence in humans that the substance can lead to specific respiratory hypersensitivity; and / or if there are positive results from an appropriate animal test. Only a single case of asthma associated to tetramethrin exposure is reported. Therefore **RAC, in concordance with the DS, considered that the evidence was not sufficient for supporting classification of d-trans-tetramethrin and concluded on no classification for respiratory sensitisation.**

10.7 SKIN SENSITISATION

Table 35: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
OECD 406, Buehler	Guinea pig, Albino, NIH (Dunkin Hartley), Vehicle: 5 M+5 F Pos.Co: 5 M+5 F Treatment group: 10 M+10 F	Tetramethrin,	0.5 g, as paste in deionised water 6 h	No animals sensitised to tetramethrin (0/20); 2-MBT control: 08/10 Not sensitising	Prakash P.J., (2006). Toxicology Department, Advinus Therapeutics Private Limited, Endura Study N° 4415/05
Buehler, pre-guideline, non-GLP	Guinea pig, Hartley, 10 M	d-Tetramethrin	50% (0.5 ml of test material diluted in acetone); Time of removal of test substance not specified	No animals sensitised to d-tetramethrin (0/10); DNCB control: 10/10 M+K test not possible due to strong irritation reaction after intradermal application Not sensitising	Hara S, Suzuki T, 1980, Sumitomo Report No. IT-00-0082

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
Buehler, sim. to OECD 406 (deficiencies)	Guinea pig, Hartley, 10 M positive control: 3 M	Tetramethrin	500 mg, applied undiluted, time of removal of test substance not specified	No animals sensitised to tetramethrin (0/10); positive control DNCB 3/3	Nakanishi T, 1990, Sumitomo Report No. IT-00-0218
Pre-guideline, non-GLP Severe deviations from M + K protocol	Guinea pig, Hartley, 7 M positive control: 5 M	Tetramethrin	1 % solution in corn oil, 10 intracutaneous injections over 23 d, challenge 14 d later	No animals sensitised to tetramethrin (0/7); Positive control DNCB 5/5 Severe deviations from M + K protocol Not sensitising	Okuno Y et al., 1976, Sumitomo Report No. IT-60-0013 Reviewed in WHO, IPCS, 1990, Environmental Health Criteria 98, Tetramethrin

Table 36: Summary table of human data on skin sensitisation

Type of data/report	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
Human patch test, modified Schwartz Peck method, pre-guideline, non-GLP	Tetramethrin	Human 23 M + 177 F, Semi-occlusive Composition of test formulation and concentration of tetramethrin not clear	No cases of sensitisation observed Not reliable, due to ethic and scientific deficiencies No conclusion possible	Osbourn R, 1966, Sumitomo Report No. IT-61-0008

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

No evidence for skin sensitisation potential of tetramethrin or d-tetramethrin was observed in a total of three Buehler tests (Prakash 2006; Hara and Suzuki, 1980; Nakanishi 1990). The test substances were applied diluted in acetone (d-tetramethrin, 0/10 animals sensitised), undiluted, apparently as solid substance (tetramethrin, 0/10 animals sensitised), and as paste in deionised water (tetramethrin, 0/20 animals sensitised). DNCB (2,4-dinitrochlorobenzene; 10/10 resp. 3/3 animals sensitised) and 2-MBT (2-mercaptobenzothiazole; 8/10 sensitised) were used as positive controls, respectively, and showed the expected skin reactions.

The application of (solid) tetramethrin to the skin in its undiluted form may have hampered skin penetration as a prerequisite for sensitisation. Nevertheless, lack of sensitising potential for tetramethrin is in line with the outcome of the test performed with d-tetramethrin. An additional study involving intracutaneous injections of tetramethrin diluted in corn oil yielded no indication for skin sensitisation (Okuno et al., 1976). However, low reliability is assigned to this study, since it shows severe deviations from the Magnusson-Kligman protocol in OEDC 406 (i. e. no use of adjuvant, no justification for selection of concentration).

A study with 200 humans (Osbourn 1966) was judged as not reliable. The substance tested was a tetramethrin formulation of unknown specification which was heated as well as autoclaved without analysing the actual tetramethrin content prior to application. Furthermore, use of the study is ethically not justifiable as no statement of informed consent of the study subjects (some of them pregnant women) was provided.

10.7.2 Comparison with the CLP criteria

Table 37: Results of skin sensitisation studies in comparison to the CLP criteria

Toxicological results	CLP criteria
No animals sensitised to tetramethrin (result of pre-guideline study with deviations from M+K protocol)	Guinea pig maximisation test Category 1A (H317): ≥ 30 % responding at ≤ 0.1 % intradermal induction dose or ≥ 60 % responding at > 0.1 % to ≤ 1 % intradermal induction dose Category 1B (H317): ≥ 30 % to < 60 % responding at > 0,1 % to ≤ 1 % intradermal induction dose or ≥ 30 % responding at > 1 % intradermal induction dose
No animals sensitised to tetramethrin or d-tetramethrin (results of 3 Buehler tests)	Buehler assay Category 1A (H317): ≥ 15 % responding at ≤ 0.2 % topical induction dose or ≥ 60 % responding at > 0.2 % to ≤ 20 % topical induction dose Category 1B (H317): ≥ 15 % to < 60 % responding at > 0.2 % to ≤ 20 % topical induction dose or ≥ 15 % responding at > 20 % topical induction dose

10.7.3 Conclusion on classification and labelling for skin sensitisation

No classification/labelling for skin sensitisation is proposed for tetramethrin or d-tetramethrin.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS proposed no classification for skin sensitisation since neither tetramethrin nor d-trans-tetramethrin caused sensitisation in any animal in 3 different Buehler tests. In these tests positive controls worked correctly.

Comments received during public consultation

One MSCA supported the no classification proposal.

Additional key elements

The table below summarises the Buehler and Magnusson & Kligman studies with tetramethrin.

Table: Summary table of animal studies on skin sensitisation of tetramethrin.

Method	Species Strain Sex N°/group	Dose levels Duration of exposure	Results	Reference
OECD TG 406 Buehler	Guinea pig Albino Vehicle: 5 M+5 F Pos.Co: 5 M+5 F Treatment group: 10 M+10 F	0.5 g as paste in deionised water 6 h	No animals sensitised to tetramethrin (0/20) 2-MBT control: 8/10	Prakash, 2006
Buehler Similar to OECD TG 406 (deficiencies)	Guinea pig Hartley 10 M Positive control: 3 M	500 mg applied undiluted Time of removal of test substance not specified	No animals sensitised to tetramethrin (0/10) Positive control DNCB 3/3	Nakanishi, 1990
Pre-guideline Non-GLP Severe deviations from	Guinea pig Hartley 7 M Positive control:	1% solution in corn oil 10 intracutaneous injections over 23 days	No animals sensitised to (0/7) Positive control DNCB 5/5	Okuno *et al.*, 1976

Magnusson & Kligman protocol	5 M	Challenge 14 days later		

Assessment and comparison with the classification criteria

The table below summarises the available Buehler study with d-trans-tetramethrin.

Table: Summary table of animal studies on skin sensitisation of d-trans-tetramethrin.

Method	Species Strain Sex N°/group	Dose levels Duration of exposure	Results	Reference
Pre-guideline Buehler Non-GLP	Guinea pig Hartley 10 M	50% (0.5 mL of test material diluted in acetone) Time of removal of test substance not specified	No animals sensitised to d-trans-tetramethrin (0/10) DNCB control: 10/10	Hara and Suzuki, 1980

No evidence of skin sensitisation potential for tetramethrin or d-trans-tetramethrin was found as no animals were sensitised in 3 different Buehler studies (the concurrent positive controls were sensitised; 80-100%). RAC notes that tetramethrin administration as a solid in the Nakanishi's study might have hampered the dermal absorption. However, the result of this study is in concordance with the other 2 Buehler studies showing no sensitisation when tetramethrin and d-trans-tetramethrin were administered, either as paste in deionised water or diluted in acetone. Despite the deviations in the protocol, 1% solution of tetramethrin in corn oil administered by intradermal injection also failed to induce sensitisation (Okuno *et al*., 1976), which supports the no sensitising potential of the substance.

The CLP Regulation establishes the substances shall be classified as skin sensitisers when they induce sensitisation in at least of 15% of exposed animals, which is a criteria that was not met in the case of tetramethrin, because no animals showed reactions after challenges. Therefore, RAC supported the DS's opinion and concluded on **no classification for skin sensitisation** for d-trans-tetramethrin**.**

10.8 GERM CELL MUTAGENICITY

Table 38: Summary table of mutagenicity/genotoxicity tests in vitro

Method, guideline, deviations if any	Test system (Organism, strain)	Test substance, reference to table 5	Concentrations tested (range)	Results		Remarks (information on cytotoxicity)	Reference
				+ S9	- S9		
Bacterial reverse mutation test OECD 471	*Salmonella typhimurium*: TA98 TA100 TA102 TA1535 TA1537	Tetramethrin, dissolved in DMSO	Pre- test: 50-5000 µg/plate Main assay: 313-5000 µg/plate	+/- TA100 Neg. for all other strains	-	TA100: two-fold increase in the number of revertant colonies (+S9), no dose-response relationship No cytotoxicity up to the highest concentration tested Outcome inconclusive	Scarcella O, 2004, Research Toxicology Centre S.p.A., Endura Study No. 9324
Ames test, pre-guideline, sim. to OECD 471, non-GLP	*S. typhimurium:* TA98 TA100 TA1535 TA1537 TA1538 E. coli: WP2 uvr A	d-Tetramethrin, dissolved in DMSO	10-5000 µg/plate	-	-	No cytotoxicity up to the highest dose tested Negative for genotoxicity	Kishida F, Suzuki H, 1980, Sumitomo report No. IT-00-0101
Bacillus subtilis rec assay, pre-guideline, sim. to OECD 471, non-GLP	*B. subtilis:* M45 rec$^-$ H17 (wild type)	d-Tetramethrin, dissolved in DMSO	1-500 mg/ml (10-5000 µg/plate)	-	-	No cytotoxicity up to the highest dose tested Negative for genotoxicity	Kishida F, Suzuki H, 1980, Sumitomo report No. IT-00-0101
Bacterial reverse mutation test Non-GLP, pre-guideline, sim. to OECD 471	*S. typhimurium:* TA1535, TA1537, TA97, TA98, TA100 *E. coli:* WP2uvrA	Tetramethrin, dissolved in DMSO	100-5000 µg/plate	-	-	Precipitations of test substance on plates at ≥ 2000 µg/plate (without S9) and at 5000 µg/plate (with S9) Cytotoxicity: TA97 (-S9): (> 500 µg/plate) Negative for genotoxicity	Kogiso S, Yoshitake A, 1987, Sumitomo Report No. IT-70-0205

DNA-repair test Non-guideline, Non-GLP	*E. coli:* W3110/polA$^+$ and p3478/polA$^-$	Tetramethrin, dissolved in DMSO	100- 33333 µg/ plate	-	-	No cytotoxicity up to the highest concentration tested Negative for genotoxicity	McGregor DB, 1984, Inveresk Research International, Endura Report No. 2988
Chromosomal aberration test OECD 473	Chinese hamster Ovary (CHO) cells	Tetramethrin, dissolved in DMSO	Experiment I: 0.781-200 µg/ml, treatment time 3 h Experiment II: 1.56-200 µg/ml, treatment time 21 h	+/-	+/-	Genotoxicity: Increase in CA at: +S9: ≥ 50 µg/mL -S9: ≥100 µg/mL (Exp. I), ≥50 µg/mL (Exp. II) Cytotoxicity: +S9: ≥ 100 µg/ml, -S9 ≥ 50 µg/ml Result: equivocal	Cilutti P, 2004, Research Toxicology Centre S.p.A., Endura Study No. 9325
Chromosomal aberration study, sim. to OECD 473	Chinese hamster ovary (CHO) cells	Tetramethrin, dissolved in ethanol	-S9: 15.1-80.3 µg/ml + S9: 50.2-151 µg/ml	+	-	Cytotoxicity: ≥ 20.1 µg/ml (-S9, 20 h, ≥ 75.3 µg/ml (+S9, 20 h) ≥ 101 µg/ml (+S9, 30h) Test considered positive under metabolic activation conditions	Murli H, 1989, Sumitomo Report No. IT-91-0216

Gene mutation assay OECD 476	Mouse lymphoma L5178Y TK$^{\pm}$ cells	Tetramethrin, dissolved in DMSO	Cytotoxicity assay: 1.56-400 µg/ml Experiment I: 1.56-40 µg/ml, treatment for 3 h Experiment II: 3.13-75 µg/ml, treatment for 3 or 24 h	+/-	-	Genotoxicity: + S9: ≥ 50µg/ml (high concentrations only tested in Exp. II, 3 h); -S9: ≥ 50 µg/ml (high concentrations only tested in Exp. II, 24 h) Cytotoxicity: +S9: 75 µg/ml (Exp. II, 3 h): -S9: ≥ 50µg/ml (Exp. II, 24 h Outcome: equivocal	Cinelli S, 2004, Research Toxicology Centre S.p.A., Endura Study No. 9326
Mutation test, sim. OECD 476, non-GLP	Chinese hamster V79 cells	Tetramethrin, dissolved in DMSO	- S9: 3.75-30 µg/ml + S9: 25-200 µg/ml	-	-	Cytotoxicity: ≥ 3.75 µg/ml (- S9), ≥ 100 µg/ml (+ S9) Test result: negative	Kogiso S, 1989, Sumitomo Report No. IT-90-0214
UDS assay, sim. to OECD 482 non-GLP	Primary rat hepatocytes	Tetramethrin, dissolved in DMSO	0.2-100 µg/ml	N/A	-	Cytotoxicity: 30-33 % viability at 100 µg/ml Test result: negative	Kogiso S, 1988, Sumitomo Report No. IT-80-0213

Table 39: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Species, strain, sex, No/group	Test substance, reference to table 5	Route and frequency of application	Dose levels and sampling times	Results	Reference
Micro-nucleus test OECD 474	Mice, Swiss albino NsdOla: MF1 5 M+5 F	Tetramethrin 200 mg/ml in vehicle (0.5% aqueous carboxymethyl celluose with Tween 80, 1 mL/L)	Oral gavage, twice at interval of 24 h	2000 mg/kg bw (Limit dose) Sampling at 24 h after 2nd treatment	Negative, At all sampling times	Badarinath J.C., (2006). Toxicology Department, Advinus Therapeutics Private Limited, Endura Study No. 4416/05
Chromosomal aberration test, sim. to OECD 475, non-GLP	Mouse, ICR, 4-6 M	d-Tetramethrin, vehicle: corn oil	Single intraperito-neal dose	Sampling times: 6, 12, 24, 48 h Doses: 150, 300 mg/kg bw (24 h), 600 mg/kg bw (all sampling times)	Negative, at all doses and sampling times	Hara M, Suzuki H, 1981, Sumitomo Report No. IT-10-0102
Chromo-somal aberration test, sim. to OECD 475	Mouse, IRC, 5 M + 5 F	Tetramethrin, Vehicle: corn oil	Single intraperito-neal dose	0-500-1000-2000 mg/kg bw Sampling 6, 18, 30 h after injection	Negative, at all doses and sampling times	Murli H, 1992, Sumitomo Report No. IT-21-0254

Table 40: Summary table of human data relevant for germ cell mutagenicity

Type of data/report	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
No data				

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

Summary of results obtained in vitro:

In general, negative results were obtained with d-tetramethrin and tetramethrin in bacterial mutation assays, irrespective of metabolic activation (Kishida and Suzuki 1980, Kogiso and Yoshitake 1987, both Sumitomo; McGregor 1984, Scarcella 2004; both Endura). Only one exception was reported in the study by Scarcella 2004, Endura, in that inconclusive results for the tester strain TA 100 were obtained with metabolic activation.

Regarding *in vitro* testing with mammalian cell systems, negative results for tetramethrin were supported by one *in vitro* test using Chinese hamster ovary (CHO) cells (Kagiso 1989; Sumitomo). None of the concentrations tested exhibited chromosomal aberrations, irrespective of metabolic activation. In addition, an UDS assay in primary rat hepatocytes (Kogiso 1988; Sumitomo) showed negative results without S9-mix (not tested with S9 mix).

By contrast, test results for tetramethrin were considered positive in a chromosomal aberrations assay in CHO cells after metabolic activation (Murli 1989; Sumitomo). Equivocal results were obtained in a further chromosomal aberration assay using CHO cells (Cilutti 2004; Endura) as well as in one mouse lymphoma assay using L5178Y $TK^{\pm}$ cells (Cinelli 2004; Endura). In the study performed by Cilutti, 2004, increases of chromosomal aberrations were observed at the highest concentrations tested with and without metabolic activation, and were accompanied by increasing cytotoxicity.

In the gene mutation assay by Cinelli, 2004, a statistically significant increase in mutant frequency was observed in the presence of S9-mix at the highest tetramethrin concentrations tested (50 and 75 µg/mL). At 50 µg/mL a 1.7-fold increase in mutant frequency was observed with a calculated survival rate of 81 %. At 75 µg/mL, mutant frequency increased to 1.9-fold of the control values but cytotoxicity was also high (57 % survival rate). As the results were observed with increasing cytotoxicity and the experiment was not repeated with the two highest concentrations, the results were regarded as equivocal. Increases of mutant frequency in the assay without metabolic activation at ≥ 50 µg/mL are not considered positive, as they were only observed at cytotoxic levels (relative survival of 25 and 0 % at 50 resp. 75 µg/mL).

In summary, there is some indication for a slight mutagenic potential of tetramethrin in *in vitro* test systems involving mammalian cells. However, as effects were observed at increasing cytotoxic dose levels, the results are regarded as equivocal.

Summary of results obtained in vivo:

Tetramethrin and d-tetramethrin showed negative results in all *in vivo* genotoxicity assays performed. Tetramethrin displayed negative results in the Mammalian Erythrocyte Micronucleus test following oral administration of 2x 2000 mg/kg bw (limit dose) at an interval of 24 hours (Badarinath 2006; Endura). The results were supported by two earlier chromosomal aberration studies with tetramethrin (Murli 1992; Sumitomo) and d-tetramethrin (Hara and Suzuki 1981; Sumitomo). In both assays no chromosomal aberrations were observed in mammalian bone marrow after single intraperitoneal application of dose levels between 150 and 600 mg/kg bw (d-tetramethrin) and between 500 and 2000 mg/kg bw (tetramethrin).

However, the negative results for tetramethrin in the micronucleus assay have to be interpreted with care, as no data for the oral bioavailability of tetramethrin were provided in the study. Moreover, vehicle effects on oral absorption cannot be excluded. Carboxymethyl cellulose was used as the dosing vehicle which has been found to decrease the absorption rate of pyrethroids (e.g. deltamethrin) and may thereby reduce the toxic potential of pyrethroid compounds (Crofton et al. 1995; USEPA, 2007). Indeed, the limit dose used in the micronucleus assay (2x 2000 mg/kg) is above the oral LD_{50} of d-tetramethrin for mice (LD_{50} ~ 1000 mg/kg; Kohda et al. 1980; Sumitomo) and no significant toxic effects were observed (Badarinath 2006; Endura). By contrast, toxic signs were observed in all dosage groups of mice (500, 1000, 2000 mg/kg bw) after single i.p. application of tetramethrin in corn oil (Murli 1992, Sumitomo). The maximal tolerated dose after single i.p. application of d-tetramethrin was 600 mg/kg (7/10 deaths at 1200 mg/kg bw; Hara and Suzuki, 1981; Sumitomo).

Consequently, the validity of the two *in vivo* studies carried out with i.p. application is regarded as being higher with respect to assessment of the genotoxic potential (negative) of tetramethrin, although effects of corn oil as vehicle for intraperitoneal application cannot be ruled out completely.

Overall, negative *in vivo* findings for chromosomal aberrations and micronucleus formations outweigh the positive test results of the above mentioned inconclusive or positive *in vitro* tests, although results of *in vivo* testing after oral application of tetramethrin can only be considered reliable if evidence is provided in the assay for the systemic bioavailability of tetramethrin after oral application.

10.8.2 Comparison with the CLP criteria

Table 41: Results of genotoxicity studies in comparison to the CLP criteria

Toxicological results	CLP criteria
Testing *in vitro*: Bacterial mutation assays: Generally negative for tetramethrin and d-tetramethrin Tests involving mammalian cells: - Negative (mutation test with CHO cells, UDS test with rat hepatocytes, tetramethrin) - Positive with S9-mix (chromosomal aberration test, tetramethrin) - Equivocal (chromosomal aberration test, mouse lymphoma cell gene mutation test, tetramethrin) Testing *in vivo* (experiments in mammals): Negative (micronucleus test and chromosomal aberration test, tetramethrin; chromosomal aberration test, d-tetramethrin)	The classification in Category 1A is based on positive evidence from human epidemiological studies. Substances to be regarded as if they induce heritable mutations in the germ cells of humans. The classification in Category 1B is based on: — positive result(s) from *in vivo* heritable germ cell mutagenicity tests in mammals; or — positive result(s) from *in vivo* somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells *in vivo*, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or — positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people. The classification in Category 2 is based on: — positive evidence obtained from experiments in mammals and/or in some cases from *in vitro* experiments, obtained from: — somatic cell mutagenicity tests *in vivo*, in mammals; or — other *in vivo* somatic cell genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assays. Note: Substances which are positive in *in vitro* mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

No human data are available for tetramethrin or d-tetramethrin, hence a classification in category 1A is not possible.

On the basis of the negative results from *in vivo* animal studies (especially after i.p. administration), no further classification or labelling of tetramethrin and d-tetramethrin for genotoxicity is proposed.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

There are some indications for a slight mutagenic potential of tetramethrin in *in vitro* test systems involving mammalian cells. However, as effects were observed at increasing cytotoxic dose levels, the results are regarded as equivocal. The negative *in vivo* findings for chromosomal aberrations and micronucleus formations outweighed the positive *in vitro* tests. All these considerations made the DS conclude on no classification of d-trans-tetramethrin for germ cell mutagenicity.

Comments received during public consultation

One MSCA supported the no classification proposal.

Additional key elements

The table below summarises the *in vitro* mutagenicity studies with tetramethrin.

Table: Results of the *in vitro* tests performed with tetramethrin.
(in all cases the substance was dissolved in DMSO)

Method	Test system	Concentrations tested	Results		Remarks	Reference
			+ S9	- S9		
OECD TG 471 Bacterial reverse mutation test	*Salmonella typhimurium:* TA98, TA100, TA102, TA1535 and TA1537	Preliminary test: 50-5000 µg/plate Main assay: 313-5000 µg/plate	+/- TA100 - for all other strains	-	TA100 (+S9): two-fold increase in the number of revertant colonies but no dose-response No cytotoxicity up to the highest concentration tested	Scarcella, 2004
Pre-guideline similar to OECD TG 471 Bacterial reverse mutation test	*Salmonella. typhimurium:* TA1535, TA1537, TA97, TA98 and TA100	100-5000 µg/plate	-	-	Precipitations of test substance on plates at ≥ 2000 µg/plate (without S9) and at 5000	Kogiso and Yoshitake, 1987

Non-GLP	*Escherichia coli:* WP2uvrA				µg/plate (with S9) Cytotoxicity: TA97 (-S9): (> 500 µg/plate)	
Non-guideline DNA-repair test Non-GLP	Escherichia coli: W3110/polA+ and p3478/polA-	100-33333 µg/ plate	-	-	No cytotoxicity up to the highest concentration tested	McGregor, 1984
OECD TG 473 Chromosomal aberration test	Chinese hamster ovary (CHO) cells	Experiment I: 0.781-200 µg/mL, treatment time 3 h Experiment II: 1.56-200 µg/mL, treatment time 21 h	+/-	+	Genotoxicity: Increase in chromosomal aberrations at: +S9: ≥ 50 µg/mL -S9: ≥ 100 µg/mL (Experiment I), ≥ 50 µg/mL (Experiment II) Cytotoxicity: +S9: ≥ 100 µg/ml, -S9 ≥ 50 µg/mL	Cilutti, 2004
Similar to OECD TG 473 Chromosomal aberration study	CHO cells	-S9: 15.1-80.3 µg/mL + S9: 50.2-151 µg/mL	+	-	Cytotoxicity: ≥ 20.1 µg/mL (-S9, 20 h) ≥ 75.3 µg/mL (+S9, 20 h) ≥ 101 µg/mL (+S9, 30h)	Murli, 1989
OECD TG 476 Gene mutation assay	Mouse lymphoma L5178Y TK ± cells	Experiment I: 1.56-40 µg/mL, treatment for 3 h Experiment II: 3.13-75 µg/mL, treatment for 3 or 24 h	+	+/-	Genotoxicity: + S9: ≥ 50 µg/mL -S9: ≥ 50 µg/mL (high concentrations only tested in Exp. II, 24 h) Cytotoxicity:	Cinelli, 2004

					+S9: 75 µg/mL (Experiment II, 3 h) -S9: ≥ 50 µg/mL (Experiment II, 24 h)	
Similar to OECD TG 476 Mutation test, Non-GLP	Chinese hamster V79 cells	- S9: 3.75-30 µg/mL + S9: 25-200 µg/mL	-	-	Cytotoxicity: ≥ 3.75 µg/mL (- S9), ≥ 100 µg/mL (+ S9)	Kogiso, 1989
Similar to OECD TG 482 UDS assay Non-GLP	Primary rat hepatocytes	0.2-100 µg/mL	N/A	-	Cytotoxicity: 30-33% viability at 100 µg/mL	Kogiso, 1988

The table below summarises the *in vivo* mutagenicity studies with tetramethrin.

Table: Results of the in vivo tests performed with tetramethrin.

Method	Species Strain Sex Nº/group	Route Frequency of application	Dose levels Sampling times	Results	Reference
OECD TG 474 Micronucleus test	Mouse Swiss albino NsdOla: MF1 5 M + 5 F	Oral gavage (Vehicle: 0.5% aqueous carboxymethyl cellulose with 1 mL/L Tween 80) Two doses at interval of 24 h	2000 mg/kg bw (200 mg/mL in vehicle) Sampling at 24 h after 2nd treatment	Negative at all sampling times	Badarinath, 2006
Similar to OECD TG 475 Chromosomal aberration test	Mouse IRC 5 M + 5 F	Single intraperitoneal dose	0-500-1000-2000 mg/kg bw Sampling 6, 18, 30 h after injection	Negative at all doses and sampling times	Murli, 1992

Assessment and comparison with the classification criteria

The table below summarises the *in vitro* mutagenicity studies with d-trans-tetramethrin.

Table: Results of the *in vitro* tests performed with d-trans-tetramethrin.
(in all cases the substance was dissolved in DMSO)

Method	Test system	Concentrations tested	Results		Remarks	Reference
			+ S9	- S9		
Pre-guideline similar to OECD TG 471 Ames test Non-GLP	*Salmonella. typhimurium*: TA98, TA100, TA1535, TA1537 and TA1538 *Escherichia coli:* WP2 uvr A	10-5000 µg/plate	-	-	No cytotoxicity up to the highest dose tested	Kishida and Suzuki, 1980
Pre-guideline similar to OECD TG 471 *Bacillus subtilis* rec assay Non-GLP	*Bacillus subtilis:* M45 rec-H17 (wild type)	1-500 mg/mL (10-5000 µg/plate)	-	-	No cytotoxicity up to the highest dose tested	Kishida and Suzuki, 1980

The table below summarises the *in vivo* mutagenicity studies with d-trans-tetramethrin.

Table: Results of the *in vivo* tests performed with d-trans-tetramethrin.

Method	Species Strain Sex N°/group	Route Frequency of application	Dose levels Sampling times	Results	Reference
Similar to OECD TG 475 Chromosomal aberration test Non-GLP	Mouse ICR 4-6 M	Single intraperitoneal dose	Sampling times: 6, 12, 24, 48 h Doses: 150, 300 mg/kg bw (24 h), 600 mg/kg bw (all sampling times)	Negative	Hara and Suzuki, 1981

An overall analysis of the information available regarding the *in vitro* genotoxicity of tetramethrin shows clear negative results (for both with and without S9) in two bacterial reverse mutation assays, one DNA repair tests in bacteria and one mutation tests in mammal cells. The negative results in bacteria were also supported by other two independent negative studies with d-trans-tetramethrin. An unscheduled DNA synthesis test in rat hepatocytes was also negative in absence of S9, no results were available in presence of S9 and one chromosomal aberration test in CHO cells was negative in absence of S9 but positive in its

presence. Several inconclusive or equivocal results were also found, e.g., one test with *Salmonella typhimurium* yielded negative results in absence of S9 and equivocal in its presence (an increase of revertant colonies in only one strain but without dose-response relationship). Equivocal results were also found in one chromosomal aberration test with hamster ovary cells and in one gene mutation assay with mouse lymphoma. In these two studies, positive results were found in only one of the two situations, either with or without S9, but the second was positive only together with a high degree of cytotoxicity. In conclusion, there are some indications of a slight mutagenic potential of tetramethrin in systems involving mammalian cells.

An overall analysis of the information available regarding the *in vivo* genotoxicity of tetramethrin shows clear negative results in one micronucleus test (oral gavage) and in one chromosomal aberration test (intraperitoneal administration), both in mouse. The negative results with tetramethrin were supported by another negative chromosomal aberration test with intraperitoneal administration of d-trans-tetramethrin in mouse. RAC noted that the limitation of the micronucleus test was the lack of information regarding bioavailability of tetramethrin, although it was remarkable that the employed dose was a limit dose around two times the LD_{50} dose estimated for d-trans-tetramethrin in mouse. In contrast, toxic signs were observed in the chromosomal aberration tests mice after the intraperitoneal administration of tetramethrin and d-trans-tetramethrin, hence it can be assumed that the substances were bioavailable.

Comparison with the criteria

Classification in Category 1A is based on positive evidence from human epidemiological studies and such studies were not available; therefore this category is clearly not supported.

Classification in Category 1B is based on positive result(s) from *in vivo* heritable germ cell mutagenicity tests in mammals; or from *in vivo* somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. RAC noted that classification for this category requires positive results in *in vivo* assays. This was not seen and therefore the criteria for classification in Category 1B were not met.

Classification in Category 2 is based on positive evidence obtained from experiments in mammals and/or in some cases from *in vitro* experiments, obtained from somatic cell mutagenicity tests *in vivo*, in mammals; or other *in vivo* somatic cell genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assays. RAC noted that positive or inconclusive *in vitro* results were found, although occurring together with other negative *in vitro* results. RAC also noted that the *in vivo* studies yielded consistent negative results and this outweighs the positive and equivocal *in vitro* results. Therefore, RAC considered that the criteria for classifying tetramethrin as a germ cell mutagen are not met and concluded, supporting the DS's proposal, that **no classification for germ cell mutagenicity is warranted.**

10.9 CARCINOGENICITY

Table 42: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
Sim. to OECD 453, Oral, dietary, 104 weeks	Mouse, B6C3F1, 50 M + 50 F, satellite group: 40 M + 40 F	Tetramethrin	0-12-60-300-1500 ppm (0-2.4/3.5-12/17-61/85-300/430 mg/kg bw/d (M/F)), 104 weeks	<u>No non-neoplastic effects</u> <u>No neoplastic effects</u>, no increased tumour rate	Cox R, 1986, Sumitomo Report No. IT-61-0193
Sim. to OECD 453, non-GLP, Oral, dietary, 104 weeks	Rat, CRL:SD:COBS, 50 M + 50 F, controls: 60 M + 60 F F1A litters from a one-generation study (animals exposed also throughout gestation and weaning)	Tetramethrin	0-1000-3000-5000 ppm (0-42/55-125/165-230/300 mg/kg bw/d (M/F) 104 weeks post weaning	<u>Non-neoplastic effects:</u> ≥ 3000 ppm: Cytoplasmic vacuolation of midzonal hepatocytes (M), testes enlarged (M), reduced bw and food consumption <u>Neoplastic effects:</u> ≥ 3000 ppm: Increased incidence of interstitial adenomas of the testis 5000 ppm: Decrease in mammary tumour incidence (F) (see Table 33a-1) Tetramethrin was administered with the diet to rats at levels of 0, 1000, 3000, and 5000 ppm for 104 weeks post-weaning. These treated rats were obtained as F_{1a} weanlings from parental animals which had been treated with the compound at levels of 0, 1000, 3000, and 6000 ppm until sexual maturity prior to mating and which continued to receive the test compound during mating and throughout the gestation and nursing period	Rutter H A, 1974, Sumitomo Report No. IT-41-0024

Method, guideline, deviations if any	Species, strain, sex, no/group	Test sub-stance, reference to table 5	Dose levels duration of exposure	Results	Reference
Sim. to OECD 453, non-GLP, Oral, dietary, 104 weeks	Rat, CR CD and Long Evans Hooded, 50 M per strain/dose group	Tetrameth rin	0-200-1000-5000 ppm (CR CD: 0-7.5-35-180 mg/kg bw/d, Long Evans Hooded: 0-8-40-205 mg/kg bw/d) Parental: 0-1000-3000-6000 ppm	Non-neoplastic effects: 5000 ppm: Reduced bw gain, slight increase in incidence of testicular degeneration with associated hypospermatogenesis or aspermatogenesis; increased weight of testis with epididymis (LE), increased absolute and relative liver weight Neoplastic effects: 5000 ppm: Statistically significant increased incidence of interstitial tumours of the testis (see Table 33a-2) Rats were exposed to the test substance maternally from conception to weaning and via diet for 104 weeks thereafter Follow-up on the results of the study by Rutter 1974	Pence, D H, 1981, Sumi-tomo Re-port No. IT-11-0097

Table 43: Supplementary Information: Incidences of testicular and mammary gland tumours (Rutter, 1974; Report No. IT-41-0024)

Parameter	Control		1000 ppm		3000 ppm		5000 ppm	
	M	F	M	F	M	F	M	F
Number of animals examined (after 2 years / interim sacrifice)	50/10	50/10	40/10	40/10	40/10	40/10	40/10	40/10
Mortality after 2 years	17/50	14/50	23/40	14/40	11/40	9/40	18/40	17/40
No. of animals with neoplasms (including interim sacrifice)	30/60	44/60	19/50	31/50	19/50	28/50	22/50	31/50
No. of animals with only benign tumours	26	41	17	26	17	23	19	24
No. of animals with malignant tumours	4	3	2	5	2	5	3	7
Interstitial cell adenoma in the testis	2/50	-	3/40	-	9/40	-	14/40	
Tumours in the mammary gland	1/50	31/50	0/40	26/40	0/40	21/40	1/40	12/40

No statistical analysis performed.

Table 44: Supplementary Information: Incidences of interstitial cell tumors in the testis (Pence, 1981, Report No. IT-11-0097)

Parameter	Control		200 ppm		1000 ppm		5000 ppm	
	CRCD	LE	CRCD	LE	CRCD	LE	CRCD	LE
Survival data at week 104	30/50	37/50	26/50	37/50	26/50	34/50	30/50	34/50

Interstitial cell tumour in the testis, unilateral	3	4	5	3	1	2	5	10
Interstitial cell tumour in the testis, bilateral	4	0	2	0	2	2	11	12
Total interstitial tumours of testes	7	4	7	3	3	4	16	22

No statistical significance reported

CRCD: Sprague-Dawley derived male rats
LE: Male rats of Long Evans hooded strain

Table 45: Summary table of human data on carcinogenicity

Type of data/report	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
No data				

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

In a chronic (2 years) oral toxicity study on tetramethrin in mice, no adverse effects (neither neoplastic nor non-neoplastic) were observed up to the highest dose of 1500 ppm (300/430 mg/kg bw/d; Cox 1986; Sumitomo).

Two pre-guideline chronic toxicity studies were performed on tetramethrin with F_{1A} rat weanlings. In the first study by Rutter (1974, Sumitomo), F_{1A} litters from a one-generation study were further exposed to tetramethrin via the diet for 104 weeks. Since parental animals had already been orally exposed to tetramethrin, it is assumed that the F_{1A} animals were exposed from conception onward (*in utero*, via milk), although the extent of exposure prior to weaning is not known. In the second study by Pence (1981, Sumitomo), which was conducted as a follow-up on the results of the Rutter study, only male animals of two rat strains (CR:CD and Long Evans Hooded) were exposed to tetramethrin from conception to two years after birth. The exposure estimates in both of the chronic studies refer to the two year dietary period.

The results of both chronic rat studies are essentially consistent: For the study by Rutter (1974), the LOAEL was set to 3000 ppm (125/165 mg/kg bw/d) for non-neoplastic effects (lower bw gain and histological abnormalities in liver) and for neoplastic effects in males. Testicular tumours were observed already at the lowest dose (1000 ppm), but achieved statistical significance at ≥ 3000 ppm (≥125 mg/kg bw/d). Apart from the dose-dependent incidence, interstitial cell adenoma occurred bilaterally only at the medium and high doses. In addition, female rats displayed a significantly decreased incidence in benign mammary tumours (i. e. fibroadenomas) at the highest tetramethrin dose (5000 ppm).

In the study by Pence, a statistically significant increase in testicular interstitial cell tumours was confirmed in both rat strains for groups receiving the high dose (5000 ppm). Furthermore, a slight increase in the incidence of testicular degeneration with hypospermatogenesis and aspermatogenesis

as compared to control rats as well as an increase in testis and liver weight were observed (group-wise incidences not reported in the original report). The NOAELs of 35 and 40 mg/kg bw/d derived for non-neoplastic and neoplastic effects in male rats are in agreement with the results of Rutter (1974). In contrast to the chronic toxicity studies performed with tetramethrin, no striking tumour-related effects were seen in a two-generation reproduction toxicity study conducted with d-tetramethrin in rats (see Pence, 1986). However, indirect parameters for hyperplasia or neoplastic effects such as testis enlargement were not specified in the two-generation study. Furthermore, oral exposure of animals to the test substance did not exceed 38 weeks (F1 generation). In the chronic (2 year) toxicity study by Rutter (1974), the time at which testicular interstitial cell tumours were suspected (from external signs) did not pre-date week 83 of dietary exposure. The inability of the two-generation toxicity study by Pence (1986) to detect tumourigenic effects may therefore also be due to an insufficient duration in individual animal exposure.

Rats appear to be particularly susceptible towards induction of Leydig cell adenomas, and exhibit a relatively high spontaneous incidence as compared to humans (Clegg et al., 1997). However, the mode of action by which tetramethrin leads to Leydig cell adenoma has not been specified. From the presented data for tetramethrin and d-tetramethrin concerning genotoxicity testing, it appears unlikely that tetramethrin acts tumourigenic via a genotoxic mechanism. A common basis for several groups of other non-genotoxic Leydig cell adenoma-inducing agents comprises disturbance of endocrine homeostasis, e. g. perturbation of the hypothalamic-pituitary-gonadal axis. Mechanisms leading to disruption of the hypothalamic-pituitary-gonadal axis in rodents may include a decrease in circulating sex (steroid) hormones, anti-estrogenic activity or enhancement of central dopamine receptor-dependent signalling (Clegg et al., 1997; ECBI/61/03 (“thought starter” for developing an agreed position on the relevance of Leydig cell tumours in rats to humans).

Limited evidence exists that one or more of these listed mechanisms may be involved in the mode of action by which tetramethrin enhances testicular interstitial cell tumour incidence in rats. Although the effect of tetramethrin on hepatic cytochrome P-450 expression has not been investigated, pyrethrins and individual synthetic pyrethroids have been identified as inducers in rat liver enzymes (Price et al., 2007; Krechniak and Wrzesniowska, 1991). Possible enzyme induction by tetramethrin would be consistent with the observation of liver weight increase in repeated-dose studies, including the study by Pence (1981). Hepatic enzyme induction would be expected to lead to enhanced androgen catabolism, resulting in a decrease in circulating androgen levels. As a consequence, androgen-dependent negative feed-back at the hypothalamic/anterior pituitary level would be deminished, and thus enhanced secretion of GnRH and LH would occur, ultimately leading to stimulation of Leydig cell proliferation.

Substances exhibiting anti-estrogenic or selective estrogen receptor modulating activity such as tamoxifen have been associated with Leydig cell tumours in mice (Clegg et al., 1997). A disruption of the negative feed-back exerted by estrogens on the hypothalamic-pituitary system, resulting in elevation of circulating LH, may provide an explanation for anti-estrogen-dependent increase in Leydig cell tumours. A possible anti-estrogenic activity of tetramethrin is indicated by the finding within the study by Rutter (1974), that occurrence of mammary tumours was reduced in the tetramethrin high dose group. In this case, tetramethrin would be mimicking effects of the selective estrogen receptor modulator tamoxifen. Furthermore, while estradiol showed a uterotrophic action in immature female rats, tetramethrin suppressed uterine weight as compared to untreated controls, also suggesting an anti-estrogenic activity (Kim et al., 2005), although an earlier study indicated that tetramethrin did not directly compete with estradiol concerning estrogen receptor binding (Kim et al., 2004).

Furthermore, for the rat, dopamine receptor agonists have been associated with Leydig cell tumours (reviewed by Clegg et al., 1997), presumably by causing a decrease in pituitary prolactin secretion. Since the number of LH receptors on rat Leydig cells is regulated by prolactin, a reduction in circulating prolactin results in a reduction in functional LH receptors and thus to a decreased stimulation of testosterone synthesis, which ultimately leads to enhanced GnRH release and stimulation of Leydig cell proliferation. This mechanism is regarded as not being relevant for the development of Leydig cell tumours in humans (Clegg et al., 1997; ECBI/61/03; ECBI/08/04 Add.4), since LH receptor density in humans appears not to depend on prolactin stimulation. Although dopaminergic system modulation by pyrethroids has been suggested (Hossain et al., 2006), it is unknown whether tetramethrin itself leads to stimulation of dopamine receptor-dependent pathways. Therefore, it is unclear whether this mechanism is relevant in tetramethrin-dependent support of Leydig cell tumour development.

In summary, several mechanisms by which tetramethrin enhances Leydig cell tumour incidence in male rats may be considered, but additional information is required to define the precise mode(s) of action and to specify whether or not these mechanisms are of relevance to human health. As long as the mode(s) of action has/have not been specified and their relevance to humans has not been ruled out, tetramethrin should be classified as a Category 2 carcinogen based on the findings in rats. This conclusion is in accordance with recommendations along the IPCS framework for analysing the relevance of a cancer mode of action for humans (Boobis et al., 2006), with ECBI/08/04 Add. 4 (Conclusions from the Specialised Experts Meeting concerning Leydig cell tumours) and ECBI/61/03 ("thought starter" for developing an agreed position on the relevance of Leydig cell tumours in rats to humans).

Table 46: Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
Rat, CRL:SD:COBS	Leydig cell tumours	Increase in Leydig cell tumours in males; Decrease in female mammary tumours	Based on microscopic evaluation at week 104, no apparent capsular invasion or metastasis of adenomas observed		Male (Decrease in female mammary tumours)	No	oral	Not specified
Rat, CR and Long Evans Hooded	Leydig cell tumours		No differenciation between adenomas and carcinomas in the report		Male	No	Oral	Not specified

10.9.2 Comparison with the CLP criteria

Table 47: Results of carcinogenicity studies in comparison to the CLP criteria

Toxicological results	CLP criteria
No data on carcinogenicity of tetramethrin or d-tetramethrin in humans, e. g. in form of epidemiological studies, are available. Two independent rat studies show evidence for a tumourigenic effect of tetramethrin in animals (rat males, 3 rat strains, statistically significant increase in interstitial tumours of the testes).	A substance is classified in Category 1 (known or presumed human carcinogens) for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished as: Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence, or Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence. The classification in Category 1A and 1B is based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived from: — human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or — animal experiments for which there is sufficient (1) evidence to demonstrate animal carcinogenicity (presumed human carcinogen). In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals.
Despite the statistically significant increase in testicular interstitial cell tumours in two independent rat studies, evidence is not considered sufficient to place tetramethrin in Category 1B. As the mode of action leading to Leydig cell tumours in rats has not been specified, relevance to humans is unclear. However, in the absence of information demonstrating non-relevance to humans, relevance is assumed by default. Based on the available animal studies, there is some uncertainty as to which extent promotion of adenomas may have occurred to malignancy (no differentiation between adenoma and carcinomas in the study by Pence).	The placing of a substance in Category 2 (suspected human carcinogens) is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived either from limited (1) evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies. […]

Toxicological results	CLP criteria
	3.6.2.2.3. Strength of evidence involves the enumeration of tumours in human and animal studies and determination of their level of statistical significance. Sufficient human evidence demonstrates causality between human exposure and the development of cancer, whereas sufficient evidence in animals shows a causal relationship between the substance and an increased incidence of tumours. Limited evidence in humans is demonstrated by a positive association between exposure and cancer, but a causal relationship cannot be stated. Limited evidence in animals is provided when data suggest a carcinogenic effect, but are less than sufficient. The terms 'sufficient' and 'limited' have been used here as they have been defined by the International Agency for Research on Cancer (IARC) and read as follows: (a) Carcinogenicity in humans The evidence relevant to carcinogenicity from studies in humans is classified into one of the following categories: — sufficient evidence of carcinogenicity: a causal relationship has been established between exposure to the agent and human cancer. That is, a positive relationship has been observed between the exposure and cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence; — limited evidence of carcinogenicity: a positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence. (b) Carcinogenicity in experimental animals Carcinogenicity in experimental animals can be evaluated using conventional bioassays, bioassays that employ genetically modified animals, and other in-vivo bioassays that focus on one or more of the critical stages of carcinogenesis. In the absence of data from conventional long-term bioassays or from assays with neoplasia as the end-point, consistently positive results in several models that address several stages in the multistage process of carcinogenesis should be considered in evaluating the degree of evidence of carcinogenicity in experimental animals. The evidence relevant to carcinogenicity in experimental animals is classified into one of the following categories: — sufficient evidence of carcinogenicity: a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites;

Toxicological results	CLP criteria
	— limited evidence of carcinogenicity: the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs. 3.6.2.2.4. Additional considerations (as part of the weight of evidence approach (see 1.1.1)). Beyond the determination of the strength of evidence for carcinogenicity, a number of other factors need to be considered that influence the overall likelihood that a substance poses a carcinogenic hazard in humans. The full list of factors that influence this determination would be very lengthy, but some of the more important ones are considered here. 3.6.2.2.5. The factors can be viewed as either increasing or decreasing the level of concern for human carcinogenicity. The relative emphasis accorded to each factor depends upon the amount and coherence of evidence bearing on each. Generally there is a requirement for more complete information to decrease than to increase the level of concern. Additional considerations should be used in evaluating the tumour findings and the other factors in a case-by-case manner. 3.6.2.2.6. Some important factors which may be taken into consideration, when assessing the overall level of concern are: (a) tumour type and background incidence; (b) multi-site responses; (c) progression of lesions to malignancy; (d) reduced tumour latency; (e) whether responses are in single or both sexes; (f) whether responses are in a single species or several species; (g) structural similarity to a substance(s) for which there is good evidence of carcinogenicity; (h) routes of exposure; (i) comparison of absorption, distribution, metabolism and excretion between test animals and humans; (j) the possibility of a confounding effect of excessive toxicity at test doses; (k) mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity. Mutagenicity: it is recognised that genetic events are central in the overall process of cancer development. Therefore evidence of mutagenic activity *in vivo* may indicate that a substance has a potential for carcinogenic effects.

10.9.3 Conclusion on classification and labelling for carcinogenicity

Classification/labelling of tetramethrin and d-tetramethrin for carcinogenicity, "Carc. 2", is proposed, based on two independent rat studies demonstrating a statistically significant increase in incidence in Leydig cell tumours in male rats, without the knowledge whether the underlying mode of action may be of non-relevance to humans.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The DS proposed classification and labelling of d-trans-tetramethrin for carcinogenicity Category 2 based on two independent rat studies demonstrating a statistically significant increase in the incidence of Leydig cell tumours in male rats.

Comments received during public consultation

Two MSCAs supported the classification proposal.

Industry released two different position papers stating that the mechanism by which tetramethrin causes tumours in Leydig cells in rats is not relevant for humans. The DS replied that the mechanism of action has not been sufficiently clarified and therefore the relevance for humans still remains unclear. Arguments from both Industry and the DS are included and discussed in the sections below.

Assessment and comparison with the classification criteria

Analysis of carcinogenicity studies

Neoplastic and non-neoplastic lesions reported in two combined chronic and carcinogenicity studies with tetramethrin in rats and one conducted in mice are summarised in the tables below.

Table: Summary table of animal studies on carcinogenicity.

Guideline Route	Species Strain Sex N°/group	Dose levels Duration of exposure	Results	Reference
Similar to OECD TG 453 Oral (dietary)	Mouse B6C3F1 50 M + 50 F	0, 12, 60, 300, 1500 ppm (0, 2.4/3.5, 12/17, 61/85-300/430 mg/kg bw/d (M/F))	No non-neoplastic effects No neoplastic effects (no increased tumour rate)	Cox, 1986

	Satellite group: 40 M + 40 F			
Similar to OECD TG 453 Non-GLP Oral (dietary)	Rat CRL:SD:COBS 50 M + 50 F Controls: 60 M + 60 F	0, 1000, 3000, 5000 ppm (0, 42/55, 125/165, 230/300 mg/kg bw/d (M/F) Tetramethrin was administered via the diet for 104 weeks post-weaning. The treated rats were obtained as F1a weanlings from parental animals which had been treated with the substance at levels of 0, 1000, 3000, and 6000 ppm until sexual maturity prior to mating and which continued to receive the test compound during mating and throughout the gestation and nursing period	<u>Non-neoplastic effects:</u> ≥ 3000 ppm: Cytoplasmic vacuolation of midzonal hepatocytes (M), testes enlarged (M), reduced bw and food consumption <u>Neoplastic effects:</u> ≥ 3000 ppm: Increased incidence of interstitial adenomas of the testis 5000 ppm: Decrease in mammary tumour incidence (F)	Rutter, 1974
Similar to OECD TG 453 Non-GLP Oral (dietary)	Rat CR CD and Long Evans (LE) Hooded 50 M per strain/dose group	0, 200, 1000, 5000 ppm (CR CD: 0, 7.5, 35, 180 mg/kg bw/d, Long Evans Hooded: 0, 8, 40, 205 mg/kg bw/d) Rats were exposed to the test substance maternally from conception to weaning and via the diet for 104 weeks thereafter	<u>Non-neoplastic effects:</u> 5000 ppm: Reduced bw gain, slight increase in incidence of testicular degeneration with associated hypospermatogenesis or aspermatogenesis; increased weight of testis with epididymis (LE), increased absolute and relative liver weight <u>Neoplastic effects:</u> 5000 ppm: Statistically significant increased incidence of interstitial tumours of the testis	Pence, 1981

Table: Incidences of testicular and mammary gland tumours in Rutter, 1974 study.

	Control		1000 ppm		3000 ppm		5000 ppm	
	M	F	M	F	M	F	M	F
Number of animals examined (after 2 years / interim sacrifice)	50/10	50/10	40/10	40/10	40/10	40/10	40/10	40/10
Mortality after 2 years	17/50	14/50	23/40	14/40	11/40	9/40	18/40	17/40
Number of animals with neoplasms (including interim sacrifice)	30/60	44/60	19/50	31/50	19/50	28/50	22/50	31/50

Number of animals with only benign tumours	26	41	17	26	17	23	19	24
Number of animals with malignant tumours	4	3	2	5	2	5	3	7
Interstitial cell adenoma in the testis	2/50	-	3/40	-	9/40	-	14/40	-
Tumours in the mammary gland	1/50	31/50	0/40	26/40	0/40	21/40	1/40	12/40

According to information provided by Industry the historical control data from the period 1976 to 1980 indicated that the incidence of testicular interstitial cell adenoma ranged from 0 to 18% for studies of 104 weeks of duration and up to 27.1% for studies of 130 weeks of duration.

Table: Incidences of interstitial cell tumours in the testis in Pence, 1981 study.

	Control		**200 ppm**		**1000 ppm**		**5000 ppm**	
	CRCD	**LE**	**CRCD**	**LE**	**CRCD**	**LE**	**CRCD**	**LE**
Survival data at week 104	30/50	37/50	26/50	37/50	26/50	34/50	30/50	34/50
Interstitial cell tumour in the testis, unilateral	3	4	2	3	1	2	5	10
Interstitial cell tumour in the testis, bilateral	4	0	2	0	2	2	11	12
Total interstitial tumours of testes	7	4	7	3	3	4	16	22

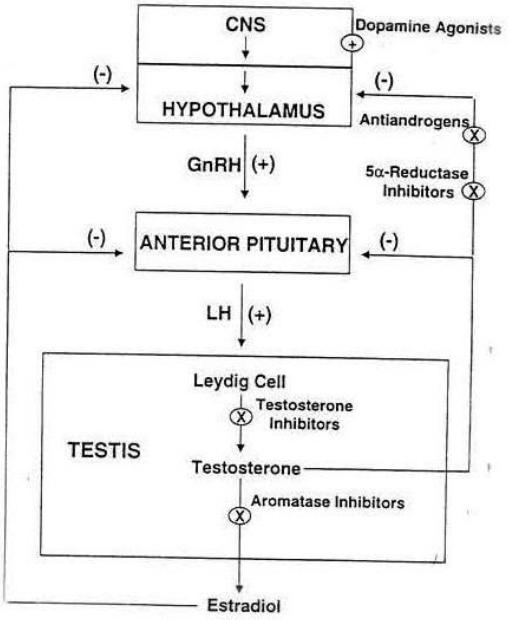
According to information provided by Industry the historical control data from the period 1981 to 1982 indicated that the incidence of testicular interstitial cell adenoma ranged from 2.3 to 12.2% and from 1983 to 1986 from 2 to 9% for studies of 104 weeks of duration.

The mouse study showed neither non-neoplastic nor neoplastic lesions. However, both rat studies showed consistent results. The Rutter (1974) rat study showed testicular tumours already at the lowest dose (42 mg/kg bw/d), but achieved a statistically significant incidence starting at the medium dose (125 mg/kg bw/d). Interstitial cell adenoma occurred bilaterally only at the medium and high doses. In the Pence (1981) study the incidence of testicular interstitial cell tumours was relevant in two different rat strains at the highest doses of 180 and 205 mg/kg bw/d. A dose-dependent reduction in the mammary gland tumour incidence was also noted in the Rutter (1974) study (incidence at the top dose was 39% compared to controls).

Mechanism of action

It is known that Leydig cell tumours are induced through impairments in the hypothalamic-pituitary-testicular axis. The figure below gives an overview of the hypothalamic-pituitary-testicular axis (Factsheets from the (eco)toxicological risk assessment strategy of the National Institute for Public Health and the Environment Part IV). The main organs involved in the regulation of testosterone concentration are the hypothalamus and the pituitary gland. The hypothalamus is involved in the secretion of gonadotropin-releasing hormone (GnRH), which stimulates the secretion of luteinizing hormone (LH) by the pituitary gland. LH binds to Leydig cells activating adenylate cyclase with the subsequent increase in cAMP levels, which stimulate testosterone biosynthesis raising testosterone levels in the bloodstream. Testosterone can be further biotransformed into estradiol and both exert a negative feedback on the release of GnRH and LH from the hypothalamus and pituitary. The overstimulation of Leydig cells by LH is one mechanism responsible of tumours.

Figure: Regulation of the hypothalamic-pituitary-testicular axis and control points for potential disruption. Symbols: (+) feedback stimulation; (-) feedback inhibition; ⊕ receptor stimulation; ⊗ enzyme or receptor inhibition (Taken from RIVM report 601516012/2004).



At least nine different modes of action based on hypothalamic-pituitary-testicular axis impairments are known for the Leydig cell tumour in rats. These mechanisms are (Rasoulpur *et al*., 2015): 1) GnRH agonism; 2) dopamine agonism/enhancement; 3) mutagenicity; 4) androgen receptor antagonism; 5) 5-alpha-reductase inhibition; 6) estrogen receptor agonism/antagonism; 7) aromatase inhibition; 8) reduced testosterone biosynthesis; and 9) increased testosterone metabolism.

From this list, only mutagenicity can be considered completely relevant for humans. Mechanisms number 1 and 2 are considered as of no relevance for humans while the rest of the proposed mechanisms are considered of low relevance for humans.

Gonadotropin-releasing hormone (GnRH) agonism

This is the mechanism proposed by Industry to explain the Leydig cell tumours found in male rats. The non-relevance of Leydig cell tumours to humans would be based on the following facts: 1) the absence of GnRH receptors in human; and, 2) the higher number of LH receptors in rat Leydig cells (around 20.000/cell) compared to human Leydig cells (around 1.500/cell). RAC considered this mechanism as plausible, but notes that no direct evidence has been provided that this mechanism is responsible of the Leydig cell tumours detected in rats.

Dopamine agonism/enhancement

This mechanism is not considered relevant for humans for the same reasons stated above since it accounts upstream to the GnRH agonism (Figure above). RAC noted that no direct

evidence was provided that this mechanism is responsible for the Leydig cell tumours detected in rats.

Mutagenicity

Mutagenicity is a mechanism that can be considered highly relevant for human carcinogenicity. However, it has been concluded that tetramethrin is not mutagenic and therefore this is not a mechanism of concern.

Androgen receptor antagonism

A potential androgen antagonism would block androgen receptors avoiding the negative feed-back of testosterone, causing a permanent release of LH that would cause Leydig cell tumours (Figure above). The CLH dossier contains results from a non-GLP rodent Hershberger assay showing no indications for androgenic or anti-androgenic effects of tetramethrin at concentrations up to 100 mg/kg bw/d. Industry pointed out that this Hershberger assay should be enough to disregard androgen receptor antagonism of tetramethrin as a cause for the Leydig cell tumours. However, RAC noted that significant carcinogenicity in the Rutter (1974) carcinogenicity assay appears at 230-300 mg/kg bw/d; while in the Pence carcinogenicity study (1981) the lowest dose inducing significant carcinogenicity was 180-204 mg/kg bw/d. Thus, RAC noted that it is unknown whether tetramethrin is able to induce androgen antagonism at tetramethrin levels causing carcinogenicity and in consequence, this mechanism, considered of low relevance for humans, cannot be ruled out.

5-alpha-reductase inhibition

5-alpha-reductase is involved in the biotransformation of testosterone into dihydrotestosterone, which binds to the androgenic receptor with greater affinity and stability than testosterone. Hence, an inhibition of 5-alpha-reductase would decrease androgenic signals received by the hypothalamus and pituitary and thereby cause a compensatory increase in LH levels, with a subsequent increase in Leydig cell tumours. The Hershberger assay confirmed incapability of tetramethrin to elicit 5-alpha-reductase activity at concentrations up to 100 mg/kg bw/d. Thus, the same limitations and concerns stated as above regarding androgen antagonism also apply and this mechanism cannot be ruled out.

Estrogen receptor agonism/antagonism

The CLH dossier contains results from an OECD rodent uterotrophic assay with tetramethrin showing that it might exert endocrine-disrupting effects on female rats through anti-estrogenic action at 5 mg/kg bw/d.

A possible anti-estrogenic activity of tetramethrin is indicated by the finding within the study by Rutter (1974), that the occurrence of mammary tumours was reduced in the tetramethrin high dose group. In this case, tetramethrin would be mimicking effects of the the selective estrogen receptor modulator tamoxifen.

This anti-estrogenic mechanism would theoretically support the hypothesis that tetramethrin would block estrogen receptors avoiding the negative feed-back and inducing excess of circulating LH with subsequent Leydig cell proliferation and tumours through excess of testosterone (Figure above). However, Industry presented a publication from the open-

scientific literature (Kim *et al*., 2004) showing that pyrethroids may be considered as estrogen-like chemicals that act through pathways other than direct endocrine receptor binding. Industry pointed out that based on this, estrogen receptor agonism/antagonism should be ruled out as a mechanism to explain the reported tumours in Leydig cells.

Aromatase inhibition

Aromatase is the enzyme involved in the conversion of testosterone into estradiol. Thus, an aromatase inhibition would result in a decrease in estradiol concentrations with subsequent increase in LH levels due to the absence of negative feed-back. Industry considers this mechanism not responsible of the Leydig tumours since it would cause an effect on ovary weight that was not detected in the one year study in dog at concentrations of 325 mg/kg bw/d (higher than carcinogenic doses in rats). RAC noted that fertility of females was not affected at concentrations of 270 mg/kg bw/d, giving additional evidences of no significant alterations in estradiol concentrations. However, RAC also noted that these are indirect evidences potentially valid for females, but it is not known if tetramethrin is causing alterations in estradiol circulating concentrations in males.

Reduced testosterone biosynthesis

Industry considered that reduced testosterone biosynthesis cannot be the mechanisms of Leydig cells tumour induction because male fertility was not affected in the reproductive toxicity studies and also because weight/appearance of prostate and seminal vesicle were not altered. RAC noted however that the CLH report does not contain information about prostate and seminal vesicle and therefore this cannot be assessed. Moreover, the absence of impairments in fertility is again indirect evidence not supported by experimental measurements of the testosterone circulating hormone after tetramethrin exposure, as would have been desirable.

Increased testosterone metabolism

Although the effect of tetramethrin on hepatic cytochrome P-450 expression has not been investigated, pyrethrins and individual synthetic pyrethroids have been identified as inducers of rat liver enzymes. Possible enzyme induction by tetramethrin and subsequent increase of biotransformation capacity would be consistent with the observation of liver weight increase in repeated-dose studies. According to Industry, steroid hormones in the rat are thought to be more susceptible to conjugation and subsequent urinary excretion compared to humans due to the lack of expression of the steroid-hormone-binding globulin in adult rats. However, it has been challenged by a publication in open scientific literature demonstrating that the expression of steroid-hormone-binding globulin is increasing with age in brain, liver and prostate (Li *et al*., 2015).

Hepatic enzyme induction would be expected to lead to enhanced androgen catabolism and together with accelerated excretion would result in a decrease in circulating androgen levels. As a consequence, androgen-dependent negative feed-back at the hypothalamic/anterior pituitary level would be diminished and thus enhanced secretion of GnRH and LH would occur, ultimately leading to stimulation of Leydig cell proliferation.

Industry has also pointed out that the increase in liver weight appeared at dose levels in rats which exceed by more that 60000-fold the typical exposure to tetramethrin for man from its use in household insecticides. RAC noted, however, that this is related to risk assessment and not to hazard identification and therefore it is not relevant for classification.

Other factors to be considered in the assessment of the tumour relevance

Industry pointed out other factors that according to them proves that the mode of action of tetramethrin for inducing tumours in Leydig cells is not relevant for humans.

1. *Histopathological assessment*

Industry undertook a re-evaluation of the histopathological diagnoses of all rats from both experiments together with an assessment of the biological significance of the findings. The histopathological examination was undertaken by the Society of Toxicologic Pathologists (Drs. S.D. Vesselinovitch and N. Ito). They classified the testicular interstitial cell lesions into one of three categories, 1) Interstitial (Leydig cell) diffuse hyperplasia, 2) Nodular hyperplasia and 3) Adenoma. Their overall interpretation from the pathology review was stated to be as follows (US EPA, 1989):

'The statistical indication of Neo-Pynamin (Tetramethrin) tumorigenicity is biologically questionable because the tumour involved is hormonally dependent, occurred at a single site, in a single sex, in a single species, and because the response to the highest dose was within the incidence range [The author is not clear about the origin of this conclusion as the data provided show that at the high dose the percentage tumour incidence is above the previously referenced historical control values] *observed in the historical controls. Since the treatment with Neo-Pynamin did not influence the development of malignant tumours at any site and because the interstitial (Leydig cell) adenomas represent a morphologic endpoint which is not associated with the malignancy, it has been concluded that the conducted bioassays did not show carcinogenic potential of Neo-Pynamin.'*

The Society of Toxicologic Pathologists has also prepared a detailed diagnostic criterion which recognises that many small focal proliferative interstitial lesions of the testis will regress following treatment withdrawal. Moreover, such adenomas in rats rarely undergo malignant transformation with progression to carcinoma.

2. *Testicular interstitial cell tumours normally occur at a much higher rate in rats than in humans.*

In experimental studies the baseline incidence of testicular interstitial cell tumours ranges from 6% in Wistar rats to as high as 100% in Fischer 344 rats. For mice, incidences range from 0.4% in the B6C3F1 strain to 1.7% in the CD-1 strain. In comparison the incidence of testicular interstitial cell tumours in humans is approximately 0.00004%.

3. *The onset of testicular interstitial cell tumours in rats is relatively late as compared to the onset in humans.*

For rats, testicular interstitial cell tumours occur primarily in aged animals whereas for humans there is an equal distribution across different age groups.

4. *Humans having certain endocrine disorders do not exhibit a high incidence of testicular interstitial cell tumours.*

Androgen Insensitivity Syndrome is a hormone-resistance disorder in which individuals have a defective androgen receptor, and Familial Male Precocious Puberty is a gonadotrophin-independent disease where a mutation in the LH receptor results in constitutive activation. The incidence of testicular interstitial cell tumours in humans with these two diseases is 2.3 and 0%, respectively.

5. *Epidemiology studies of other chemicals known to induce testicular interstitial cell tumours in rats provide no correlation in humans.*

Separate rat studies on 1,3-butadiene, cadmium, lactose, nicotine, and trichloroethylene all resulted in the appearance of rat testicular interstitial cell tumours. However, when human populations with known exposure to these chemicals were examined, no association between exposure and the induction of testicular interstitial cell tumours was observed. In addition, several currently marketed drugs (e.g. cimetidine, flutamide, bicalutamide, ketoconazole) have produced testicular interstitial cell adenomas in rodents, but no such association has been observed in humans. Moreover, the induction of interstitial cell tumours in rats by simply including 20% lactose in the diet is remarkable due to the widespread consumption of lactose by the human population with no reports to date of any association between lactose consumption and the occurrence of interstitial cell tumours in humans.

Additional considerations for classification

The CLP guidance establishes certain important factors which may be taken into consideration when assessing the overall level of concern. These factors are displayed and discussed in the table below.

Table: Some important factors which may be taken into consideration when assessing the overall level of concern of the tumours.

Tumour type and background incidence:	Leydig cell tumours
Multi-site responses:	No, only appear in testis
Progression of lesions to malignancy:	No, tumours are only benign
Reduced tumour latency:	No, the tumours occurred at a later stage of the study
Whether responses are in single or both sexes:	Single sex (males)
Whether responses are in a single species or several species:	Single species (rats)
Structural similarity to a substance(s) for which there is good evidence of carcinogenicity:	Not noted
Routes of exposure:	Oral (relevant for human)
Comparison of absorption, distribution, metabolism and excretion between test animals and humans:	Not known
The possibility of a confounding effect of excessive toxicity at test doses:	No, tumours appear in concurrence with mild not related non-neoplastic effects, well below the maximum tolerable dose

Mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity:	Potentials modes of action have been discussed above

Comparison with the criteria

A substance should be classified as carcinogenic Category 1A when it is known to have carcinogenic potential on the basis of human evidence. There is no information about the potential carcinogenicity of d-trans-tetramethrin for humans and therefore Category 1A is not supported.

A substance can be classified as carcinogenic Category 1B when it is presumed to have carcinogenic potential in humans on the basis of human evidences, while Category 2 is reserved for substances suspected to be carcinogenic on the basis of evidence not sufficiently convincing to classify as Category 1.

RAC noted that, despite the statistically significant increases in testicular interstitial cell tumours in two independent rat studies, the evidences are not strong enough to place d-trans-tetramethrin in Category 1B because there are uncertainties related to the mode of action and the relevance for humans.

RAC however considered that not all potential modes of action without relevance in humans can be disregarded with the available information and hence the relevance to humans cannot be ruled out.

In conclusion, RAC supported the DS's proposal for classifying d-trans-tetramethrin for **Carcinogenicity Category 2 (H351: Suspected of causing cancer).**

10.10 REPRODUCTIVE TOXICITY

10.10.1 Adverse effects on sexual function and fertility

Table 48: Summary table of animal studies on adverse effects on sexual function and fertility

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
Pre-guideline Sim. to OECD 415 non-GLP One-generation.	The test substance was not administered to maternal animals beyond implantation (day 7 of pregnancy), and was not administered throughout	Rat; Slc:SD 20 male and 20 female per dose group	Tetramethrin (Sumitomo) in 0.5% sodium carboxymethylcellulose (vehicle)	0, 100, 300 or 1000 mg/kg bw/d gavage Dosing started at 6 weeks of age (males) or 11 weeks (females). Continued to confirmation of mating success	**Parental:** 1000 mg/kg bw/d: Increase in liver weight (M), lower body weight gain after tetramethrin withdrawal (F)	Sato T, Tagewa G, Narama K, (1980), Sumitomo Report No. IT-01-0075

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
Repro-duction test of Neo-Pynamin, Part 1: Fertility study in rats,	pregnancy and nursing periods. Dosing of males started 9 weeks prior to the mating period. Dosing of females commenced 2 weeks prior to the mating period.			(males) or day 7 of pregnancy (females).	**Reproduc-tive:** <u>1000 mg/kg bw/d:</u> Delayed mating, lower numbers of corpora lutea and implanta-tion sites **Offspring** (F1) 1000 mg/kg bw/d: Number of surviving fetuses significantly reduced; number of corpora lutea and number of implantations reduced, lower pup weight and body length, delayed ossification **Parental and offspring NOAEL** 300 mg/kg b w/d	
Preguidelin e Sim. to OECD 415 Non-GLP		Rat Slc: SD (SPF) Male (for mating) and 11-13 (pretest)/20 (main study) females	Tetramethrin in 0.5% carboxymeth ylcellulose	100, 300 and 1000 mg/kg bw/d gavage day 7 of pregnancy to day 21 of lactation	300 and 1000 mg/kg bw: <u>Dams:</u> slight liver swelling and statisti-cally sign. increased absolute liver weight at autopsy at weaning. <u>Offspring</u>: : Sign. increase of stillborn pups, post-implantation loss in the	Sato T, Tagawa G and Narama K, (1980), Reproduction test of Neo-Pynamin, Part 4: Perinatal and postnatal study in rats, Sumitomo Report No. IT-01-0078

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
					high-dose group in pre-study No embryotoxic effects in main study Maternal NOAEL: 300 mg/kg bw/d Offspring NOAEL: 100 mg/kg bw/d	
Two-generation repro-duction toxicity Sim. to OECD 416, non-GLP	Sperm parameters not determined, longer pre-mating dose period	Rat, Sprague-Dawley, 13 male + 26 female (F_0) 15 male + 26 female (F_1)	d-Tetra-methrin	0-100-500-3000 ppm 0, 7, 35, 210 mg/kg bw/d (M) 0, 9, 45, 270 mg/kg bw/d (F) Duration of exposure not mentioned	<u>3000 ppm:</u> Parental: Reduced bw gain (F: F0/F1) during all stages (F1): Bile duct hyperplasia in maternal females Offspring (F1/F2): Reduced body weight gain during lactation (M/F) **Parental and offspring** NOAEL: 500 ppm **NOAEL reproductive :** 3000 ppm	Pence D (1986) Two generation reproduction study in rats, Sumitomo Report No. IT-61-0201
Pre-guide-line, non-GLP One-generation repro-	Suppl. study with deficiencies, e.g. parental weight development after pre-mating, pathology/histo-pathology not	Rat, Sprague-Dawley, 15 male + 30 female	Tetramethrin	0, 1000, 3000, 6000 ppm 0, 65, 185, 390 mg/kg bw/d (M)	Parental: None Offspring (F1): <u>≥ 3000 ppm:</u>	Rutter H A (1974) One generation reproduction study – Rats, Sumitomo Report No. IT-

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
duction toxicity	reported	per dose group Controls: 20 males + 40 females		0, 75, 227,482 mg/kg bw/d (F) Dietary administration Duration of exposure not mentioned.	Pup weight at weaning reduced 6000 ppm: Lower lactation index **NOAEL reproductive :** 1000 ppm	41-0042

Table 49: Summary table of human data on adverse effects on sexual function and fertility

Type of data/report	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
No data				

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

Of the submitted rat reproduction toxicity studies, one represents a two-generation study on d-tetramethrin (Pence, 1986; Sumitomo). No effects on reproductive performance were observed within this study up to the highest dose tested (3000 ppm, 210/270 mg/kg bw/d). The parental (maternal) NOAEL of 500 ppm (35/45 mg/kg bw/d) was based on reduced body weight gain in maternal animals and on bile duct hyperplasia in maternal F1 females. The offspring toxicity NOAEL of 45 mg/kg bw/d was based on reduced body weight gain in F1 and F2 generations during lactation and after feeding of 3000 ppm to maternal animals.

The other rat studies represented one-generation studies conducted with tetramethrin, administered orally either by gavage (Sato et al., 1980; Report No. 01-0075 and 01-0078, Sumitomo) or with the diet (Rutter, 1974; Sumitomo). Lower numbers of *corpora lutea* and of resulting implantations/live foetuses were reported in females at the highest dose of 1000 mg/kg bw/d in the one-generation study (01-0075), which may point to a tendency for inhibition of ovulation. However, this effect was slight (about 10 % difference) and occurred only at the highest dose applied. A reduction in litter size is not confirmed by read-across from the two-generation study for d-tetramethrin (Pence, 1986; Sumitomo). Delayed ossification in offspring in this study is attributed to reduced growth and appears to be secondary in nature, since tetramethrin was not administered to maternal animals beyond day 7 of gestation. Beside slight liver swelling and increases of liver weight as maternal toxicity at 300 and 1000 mg/kg bw/, no embryotoxic effects were seen in the second 1-generation study (01-0078).

The study conducted by Rutter (1974) is considered supplemental only. Due of lack of detail to the study report, it is unknown whether parental effects might have occurred. For example, weight development of parental animals was not recorded after the pre-mating phase and pathology/histopathology was not performed. Due to lack of detail to study, the assignment of a parental NO(A)EL is impeded. Anyhow, male and female pup weights at weaning was significantly lower in the 3000 and 6000 ppm groups. At 6000 ppm lactation index (number of pups weaned / number of pups left to nurse) was reduced.

In summary, the available data does not provide evidence for toxic effects of tetramethrin on fertility below doses causing maternal toxicity.

Comparison with the CLP criteria

Table 50: Results of studies on sexual function and fertility in comparison to the CLP criteria

Toxicological results	CLP criteria
	Category 1A: Known human reproductive toxicant Category 1B: Presumed human reproductive toxicant largely based on data from animal studies - clear evidence of an adverse effect on sexual function and fertility in the absence of other toxic effects, or - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects Category 2: Suspected human reproductive toxicant - some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility - where the evidence is not sufficiently convincing to place the substance in Category 1 (deficiencies in the study). - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects

10.10.3 Adverse effects on development

Table 51: Summary table of animal studies on adverse effects on development

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
Teratogenicity study in the rabbit OECD 414 GLP	Loss of a data book concerned with logging the quantities of test article used. This deviation does not affect the interpretation of this study.	Rabbit, New Zealand white, 20/21 females per dose group	Tetramethrin in 0.5% Carboxymethylcellulose	0, 30, 100, 300, 500 mg/kg bw/d gavage day 7-19 of gestation	In a range finding study (500, 1000, 1500 mg/kg bw/d) lack of bw gain (≥ 500 mg/kg bw/d), deaths (1, 4, 1 from 500 mg/kg bw/d onwards and abortions (2, 4, 5 from 500 mg/kg bw/d onwards) in females NOAEL maternal: 300 mg/kg bw/d	Robinson K, Washer G and Noveroske J W, (1991) An oral teratology study of Neo-Pynamin in the Rabbit, Sumitomo Report No. IT-11-0234

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
					NOAEL developmental: 500 mg/kg bw/d	
Teratogenicity study in the rat OECD 414 GLP	None	Rat, Crl:COBS VAF CD(SD)BR 25 females per dose group	Tetramethrin in 0.5% Carboxymethylcellulose	0, 150, 500, 1000 mg/kg bw gavage day 6-15 of gestation	No treatment-related effects NOAEL maternal/developmental: 1000 mg/kg bw/d	Robinson K, Washer G and Noveroske J W, (1991), An oral teratology study of Neo-Pynamin in the Rat, Sumitomo Report No. IT-11-0241
Preguideline Sim. to OECD 414 Non-GLP		Rat Slc: SD (SPF) rats 11-13 females (preliminary test), 20 females (main study)	Tetramethrin in 0.5% Carboxymethylcellulose	0, 100, 300, 1000 mg/kg bw gavage day 7 of pregnancy to day 21 of lactation	Slightly lower food consumption and higher liver weights at 1000 mg/kg bw/d NOAEL maternal 300 mg/kg bw/d NOAEL developmental: 1000 mg/kg bw/d	Sato T, Tagawa G and Narama K (1980), Reproduction test of Neo-Pynamin, Part 4: Perinatal and postnatal study in rats, Sumitomo Report No. IT-01-0078
Preguideline Sim. to OECD 414 GLP	Subcutaneous administration of the test compound, number of animals insufficient, reporting lacks details on visceral examinations of foetuses	Rabbit New Zealand white 11 females per dose group	d-Tetra-methrin in corn oil	0, 30, 100, 300 mg/kg bw/d s.c. injection once daily day 6-18 of gestation	In a range finding study (3 pregnant dams), daily dosing on days 6-18 resulted in all dams dying at 1000 and 2000 mg/kg bw/d. At 500 mg/kg bw/d lower maternal bw, lesser food consumption, and lower number of live foetuses.	Satoh R., Kashima M., Takahashi M. and Satoh H.(1982), Teratogenicity study of Neo-Pynamin Forte in rabbits, Sumitomo Report No IT-11-0142

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
					No effects at 250 mg/kg bw/d. Main study: No effects in dams and offspring. NOAEL maternal/ developmental: 300 mg/kg bw/d	
Preguideline Sim. to OECD 414 GLP	Subcutaneous administration of d-Tetramethrin. 23 dams for examination at term (day 21) and 14 dams for examination of offspring at weaning. Additional groups in which post natal development was examined	Rat Sprague Dawley 37 females per dose group	d-Tetra-methrin in corn oil	0, 100, 300, 1000 mg/kg bw/d s.c. injection once daily day 7-17 of gestation	1000 mg/kg bw/d: clonic convulsions, lower food consumption and decreases in bw, increases in liver and kidney weights of dams. 300 mg/kg bw/d: Lower food consumption No treatment-related effects in fetuses. NOAEL maternal: 300 mg/kg bw/d NOAEL developmental: 1000 mg/kg bw/d	Satoh R., Kashima M., Takahashi M. and Satoh H.(1982), Teratogenicity study of Neo-Pynamin Forte in rats, Sumitomo Report No IT-11-0141
Preguideline Sim. to OECD 414,		Rat, SD (SPF) 30 females per dose group (20	Tetramethrin in 0.5% carboxy-methyl-cellulose	0, 100, 300, 1000 mg/kg bw/d gavage day 7-17 of gesta-tion	1000 mg/kg bw/d: Lower bw gain; liver: swelling, weight increase of	Sato T and Narama K, (1980b), Reproduction Test of Neopynamin Part 2: Teratology study in rats,

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
non-GLP		females: Sacrifice at Caesarean section, 10 females sacri-fice at wean-ing)			liver and kidney. No treatment-related effects in fetuses. NOAEL maternal: 300 mg/kg bw/d NOAEL developmental: 1000 mg/kg bw/d	Sumitomo Report No. IT-01-0076
Preguideline, Sim. to OECD 414, non-GLP		Rabbit, Japanese White, 10 females per dose group Range-finding study: 6 females per dose group	Tetramethrin in 0.5% carboxy-methyl-cellulose	0, 50, 150, 500 mg/kg bw/d (main study) (range finding study: 0, 150, 500, 1500 mg/kg bw/d) gavage, day 6-18 of gestation	Range-finding study at 1500 mg/kg bw/d: In dams, decreased bw gain 500 and 1500 mg/kg bw/d: Liver weight increase Main study at 500 mg/kg bw/d: In dams, decreased bw gain Main study at 500 mg/kg bw/d: Lower bw of fetuses Skeletal anomalies (stat. not significant: 3 litter (1 foetus each) with different anomalies at 500 mg/kg bw/d)	Sato T and Narama K, (1980c), Reproduction Test of Neopynamin Part 3: Teratology study in rabbits, Sumitomo Report No. IT-01-0077

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
					NOAEL maternal / developmental: 150 mg/kg bw/d	
Preguide-line, non-GLP		Rabbit, New Zealand white, 9 females per dose group	Tetramethrin in corn oil Dosing lower than for expected effects	0-30-90 mg/kg bw/d (in add. 90 mg/kg bw/d Pyrthrin) Oral, capsule, day 8-16 of gestation	No treatment-related critical effects NOAEL maternal/developmental: 90 mg/kg bw/d	Dudeck T (1978), Reproduction Study Neo-Pynamin and Pyrethrin – Rabbits, Sumitomo Report No. IT-61-0009

Table 52: Summary table of human data on adverse effects on development

Type of data/report	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 53: Summary table of other studies relevant for developmental toxicity

Type of study/data	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
OECD rodent uterotrophic and Hershberger assays Non-GLP	Tetramethrin in corn oil	Uterotrophic assay administration was subcutaneous injection Hershberger assay administration was oral gavage Rats Sprague-Dawley, Number of animals not specified. 5 to 800 mg/kg in the uterotrophic assay 10, 50 or 100 mg/kg in the Hershberger assay	Tetramethrin may exert endocrine-disrupting effects on female rats through anti-estrogenic action. No indications for androgenic or antiandrogenic effects	Kim, S. S. et al. (2005) Assessment of estrogenic and androgenic activities of teramethrin in vitro and in vivo assays. Journal of Toxicology and Environmental Health, 68: 2277-2289.
Soto's E-screen assay (estrogen receptor affinity), Non-guideline Non-GLP	Tetramethrin	In vitro MCF-7 BUS human breast cancer cells	No evidence for estrogenic activity or estrogen receptor affinity	Kim Y. et al. (2004), Assessing Estrogenic Activity of Pyrethroid Insecticides Using In Vitro Combination assays. Journal of Reproduction and development, 50 (2): 245-255

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

From pre-guideline developmental toxicity studies in rabbits subcutaneously exposed to d-tetramethrin (Satoh et al., 1982b and respective range-finding study 1982c), a maternal and fetal NOAEL of 300 mg/kg bw/d was defined, based on mortality of all pregnant dams at 1000 and 2000 mg/kg bw/d daily subcutaneous dosing. At 500 mg/kg bw/d lower maternal body weight, lesser food consumption, and lower number of live foetuses were noted. Subcutaneous dosing of 250 mg/kg bw/d d-tetramethrin resulted in no effects. In the main study daily subcutaneous injections of 0, 30, 100, 300 mg/kg bw/d d- tetramethrin during days 6-18 of gestation no toxicological effects were seen in dams and in the offspring.

Abnormal kidney position was observed in a foetus in the 30 mg/kg group and in 3 foetuses in the 300 mg/kg group but the frequencies were not of statistical significance and therefore considered not *substance-related.* Therefore, the NOAEL (maternal / developmental) was set at 300 mg/kg bw/d.

Two further studies involving tetramethrin administration to rabbits via the oral (gavage) route support a LOAEL of 500 mg/kg bw/d for maternal toxicity and a NOAEL of 300 mg/kg bw/d. In the study by Robinson et al., (1991 a), maternal animals exhibited weight loss at this dose level, one death and two abortions occurred at 500 mg/kg bw/d in the corresponding dose range-finding study. In the

study by Sato and Narama (1980c), decreased body weight gain and an increase in liver weight were observed for dams at 500 mg/kg bw/d.

In the studies by Robinson et al. (1991a, 1991, Sumitomo), oral administration of tetramethrin revealed no developmental toxicity up to 500 mg/kg bw/d and 1000 mg/kg bw/d. Likewise, administration of 1000 mg/kg/day resulted in no maternal toxicity.

A statistically non-significant increase in the incidence of rabbit foetal skeletal anomalies was noted at 500 mg/kg bw/d tetramethrin per os (Sato and Namara, 1980c) which were not observed in the control group. As the study was not performed according to GLP and OECD guidelines (e.g. 10 animals per group only) and due to insufficient reporting and recording the study cannot be regarded as a key study. However, the results are regarded as supportive and the NOAEL of 150 mg/kg bw/d is used to establish the lowest NOAEL for developmental toxicity since it cannot be excluded that the rabbit strain used (Japanes White) is the most sensitive. In addition, the study supports the fetal LOAEL demonstrated for subcutaneously administered d-tetramethrin. Thus, from the developmental toxicity studies performed in rabbits, an overall NOAEL of 300 mg/kg bw/d was established for maternal toxicity whereas the lowest NOAEL for developmental toxicity was established at 150 mg/kg bw/d (Sato and Namara, 1980c) of tetramethrin/d-tetramethrin in the rabbit.

Four rat developmental toxicity studies were compared, one involving subcutaneous administration of d-tetramethrin and three dealing with orally administered tetramethrin. In the pre-guideline study with subcutaneous d-tetramethrin (Satoh et al., 1982a), clonic convulsions, indications for liver and kidney toxicity, as well as reduced body weight gain were reported for the dams at 1000 mg/kg bw/d, yielding a maternal NOAEL of 300 mg/kg bw/d.

Although no substance-related maternal toxicity was observed up to 1000 mg/kg bw/d in two of the studies with oral tetramethrin (Robinson et al., 1991b; Sato et al., 1980), lower body weight gain and liver/kidney weight increase at this dose in the study by Sato and Narama (1980b) are in line with the LOAEL of 1000 mg/kg bw/d set for subcutaneous d-tetramethrin. Thus, an overall maternal NOAEL of 300 mg/kg bw/d for d-tetramethrin/tetramethrin was established in rats, which is in accordance with the rabbit NOAEL for maternal toxicity.

In all of the four rat developmental toxicity studies, no treatment-related embryotoxic or teratogenic effects were noted up to the highest dose of 1000 mg/kg bw/d. Thus, the developmental NOAEL was set at 1000 mg/kg bw/d for rats, which is higher than the developmental toxicity NOAEL established for rabbits (300 mg/kg bw/d), indicating that susceptibility towards fetal toxicity may differ between species.

Evidence for an antiestrogenic activity of tetramethrin at doses of 5 mg/kg bw/d and above in female Sprague Dawley rats was provided by Kim, S.S. et al. (2005). However, antagonism to oestrogenic actions may not be mediated by binding of tetramethrin to oestrogen receptors, but rather be functional or indirect in nature as tetramethrin appeared not to compete with estradiol for estrogen receptor binding (Kim, I. Y. et al., 2004).

10.10.5 Comparison with the CLP criteria

Table 54: Results of developmental toxicity studies in comparison to the CLP criteria

Toxicological results	CLP criteria
No human data are available for tetramethrin or d-tetramethrin, hence a classification in category 1A is not possible. On the basis of the absence of embryotoxic effects and effects on sexual function and fertility especially below maternal toxicity, no classification or labelling of tetramethrin and d-tetramethrin for reproductive toxicity is proposed.	Category 1A: Known human reproductive toxicant Category 1B: Presumed human reproductive toxicant largely based on data from animal studies - clear evidence of an adverse effect on development in the absence of other toxic effects, or - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects Category 2: Suspected human reproductive toxicant - some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on development and - the evidence is not sufficiently convincing to place the substance in Category 1 (deficiencies in the study). - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects

10.10.6 Adverse effects on or via lactation

Table 55: Summary table of animal studies on effects on or via lactation

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
Refer to reproductive studies with oral application						

Table 56: Summary table of human data on effects on or via lactation

Type of data/report	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
No data				

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

Refer to reproductive studies with oral (dietary or gavage) application

10.10.8 Comparison with the CLP criteria

No data

10.10.9Conclusion on classification and labelling for reproductive toxicity

No human data are available for tetramethrin or d-tetramethrin, hence a classification in category 1A is not possible.

On the basis of the absence of embryotoxic effects and effects on sexual function and fertility especially below maternal toxicity, no further classification or labelling of tetramethrin and d-tetramethrin for reproductive toxicity is proposed.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification or labelling of d-trans-tetramethrin for reproductive toxicity based on the absence of embryotoxic effects and effects on sexual function and fertility, especially below maternally toxic doses.

Comments received during public consultation

One MSCA supported the no classification proposal.

Additional key elements

Sexual function and fertility

The table below provides an overview of the reproductive toxicity related findings in the studies performed with tetramethrin.

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

Test guideline Deviation	Species Strain Sex Nº/group	Dose levels Duration of exposure	Results	Reference

Pre-guideline Similar to OECD TG 415 Non-GLP One-generation The test substance was not administered to maternal animals beyond implantation (day 7 of pregnancy), and was not administered throughout pregnancy and nursing periods Dosing of males started 9 weeks prior to the mating period Dosing of females commenced 2 weeks prior to the mating period	Rat Slc:SD 20 male and 20 female per dose group	0, 100, 300 or 1000 mg/kg bw/d Gavage Duration of exposure not mentioned	**Parental:** 1000 mg/kg bw/d: Increase in liver weight (M), lower body weight gain after tetramethrin withdrawal (F) **Reproductive:** 1000 mg/kg bw/d: Delayed mating, lower numbers of corpora lutea and implantation sites **Offspring (F1):** 1000 mg/kg bw/d: Number of surviving fetuses significantly reduced; number of corpora lutea and number of implantations reduced, lower pup weight and body length, delayed ossification **Parental and offspring NOAEL 300 mg/kg bw/day**	Sato, 1980
Pre-guideline Similar to OECD TG 415 Non-GLP	Rat Slc: SD (SPF) Male (for mating) and 11-13 (pretest)/20 (main study) females	100, 300 and 1000 mg/kg bw/d Gavage Day 7 of pregnancy to day 21 of lactation	**Dams:** 300 and 1000 mg/kg bw/d: slight liver swelling and statistically sign. increased absolute liver weight at autopsy at weaning **Offspring:** increase of stillborn pups, postimplantation loss in the high-dose group in the pre-study No embryotoxic effects in main study **Maternal NOAEL: 300 mg/kg bw/day** **Offspring NOAEL: 100 mg/kg bw/day**	Sato *et al*., 1980

Pre-guideline One-generation reproduction toxicity Non-GLP Suppl. study with deficiencies, e.g. parental weight development after pre-mating, pathology/histopathology not reported	Rat Sprague-Dawley 15 male + 30 female per dose group Controls: 20 males + 40 females	0, 1000, 3000, 6000 ppm (0, 65, 185, 390 mg/kg bw/d (M)) (0, 75, 227,482 mg/kg bw/d (F)) Dietary administration Duration of exposure not mentioned	**Parental:** None **Offspring (F1):** <u>≥ 3000 ppm:</u> Pup weight at weaning reduced <u>6000 ppm:</u> Lower lactation index **NOAEL reproductive: 1000 ppm**	Rutter, 1974

<u>Development</u>

The table below provides an overview of the developmental toxicity-related findings in the studies performed with <u>tetramethrin</u>.

Table: Summary table of animal studies on adverse effects on development of tetramethrin.

Test guideline **Deviations**	**Species** **Strain** **Sex** **Nº/group**	**Dose levels** **Duration of exposure**	**Results**	**Reference**
OECD TG 414 Teratogenicity study in the rabbit GLP Loss of a data book concerned with logging the quantities of test article used. This deviation does not affect the interpretation of this study	Rabbit NZW 20/21 females per dose group	0, 30, 100, 300, 500 mg/kg bw/d Gavage Day 7-19 of gestation	In a range finding study (500, 1000, 1500 mg/kg bw/d) lack of bw gain (≥ 500 mg/kg bw/d), deaths (1, 4, 1 from 500 mg/kg bw/d onwards and abortions (2, 4, 5 from 500 mg/kg bw/d onwards) in females **NOAEL maternal: 300 mg/kg bw/d** **NOAEL developmental: 500 mg/kg bw/d**	Robinson *et al*., 1991
OECD TG 414 Teratogenicity study in the rat	Rat Crl:COBS VAF CD(SD)BR	0, 150, 500, 1000 mg/kg bw/d	No treatment-related effects	Robinson *et al*., 1991

GLP	25 females per dose group	Gavage Day 6-15 of gestation	**NOAEL maternal/developmental: 1000 mg/kg bw/d**	
Pre-guideline Similar to OECD TG 414 Non-GLP	Rat Slc: SD (SPF) 11-13 females (preliminary test), 20 females (main study)	0, 100, 300, 1000 mg/kg bw/d Gavage Day 7 of pregnancy to day 21 of lactation	Slightly lower food consumption and higher liver weights at 1000 mg/kg bw/d **NOAEL maternal 300 mg/kg bw/d** **NOAEL developmental: 1000 mg/kg bw/d**	Sato *et al*., 1980
Pre-guideline Similar to OECD TG 414 Non-GLP	Rat SD (SPF) 30 females	0, 100, 300, 1000 mg/kg bw/d	<u>1000 mg/kg bw/d:</u> Lower bw gain; liver: swelling, weight increase of liver and kidney. No treatment-related effects in fetuses. **NOAEL maternal: 300 mg/kg bw/d** **NOAEL developmental: 1000 mg/kg bw/d**	Sato and Narama, 1980b
Pre-guideline, Similar to OECD TG 414 Non-GLP	Rabbit Japanese White 10 females per dose group Range-finding study: 6 females per dose group	0, 50, 150, 500 mg/kg bw/d (main study) (range finding study: 0, 150, 500, 1500 mg/kg bw/d) Gavage Day 6-18 of gestation	<u>Range-finding study</u> 1500 mg/kg bw/d: In dams: decreased bw gain 500 and 1500 mg/kg bw/d: Liver weight increase <u>Main study</u> 500 mg/kg bw/d: In dams: decreased bw gain In foetus: Lower bw Skeletal anomalies (statistically not significant: 3 litter (1 foetus each) with different anomalies at 500 mg/kg bw/d) **NOAEL maternal/developmental: 150 mg/kg bw/d**	Sato and Narama, 1980c
Pre-guideline Non-GLP	Rabbit, NZW	0, 30, 90 mg/kg bw/d (in add. 90	No treatment-related critical effects	Dudeck, 1978

	9 females per dose group	mg/kg bw/d Pyrthrin) Oral (capsule) Day 8-16 of gestation	**NOAEL maternal/developmental: 90 mg/kg bw/d**	

Assessment and comparison with the classification criteria

The table below provides an overview of the reproductive toxicity related findings in the studies performed with d-trans-tetramethrin.

Table: Summary table of animal studies on adverse effects on sexual function and fertility of d-trans-tetramethrin.

Test guideline Deviations	Species Strain Sex N°/group	Dose levels Duration of exposure	Results	Reference
Similar to OECD TG 416 Two-generation reproduction toxicity Non-GLP Sperm parameters not determined, longer pre-mating dose period	Rat Sprague-Dawley 13 male + 26 female (F0) 15 male + 26 female (F1)	0, 100, 500-3000 ppm (0, 7, 35, 210 mg/kg bw/d (M)) (0, 9, 45, 270 mg/kg bw/d (F)) Duration of exposure not mentioned	3000 ppm: Parental: Reduced bw gain (F: F_0/F_1) during all stages (F_1): Bile duct hyperplasia in maternal females Offspring (F_1/F_2): Reduced body weight gain during lactation (M/F) **Parental and offspring NOAEL: 500 ppm** **NOAEL reproductive: 3000 ppm**	Pence, 1986

Development

The table below provides an overview of the developmental toxicity-related findings in the studies performed with d-trans-tetramethrin.

Table: Summary table of animal studies on adverse effects on development of d-trans-tetramethrin.

Test guideline Deviations	Species Strain Sex N°/group	Dose levels Duration of exposure	Results	Reference
Pre-guideline Similar to OECD TG 414	Rabbit NZW	0, 30, 100, 300 mg/kg bw/d s.c. injection once daily on	In a range finding study (3 pregnant dams), daily dosing on days 6-18 resulted in all dams dying at 1000 and 2000 mg/kg	Satoh *et al.*, 1982

GLP Subcutaneous administration of the test compound, number of animals insufficient, reporting lacks details on visceral examinations of foetuses	11 females per dose group	day 6-18 of gestation	bw/d. At 500 mg/kg bw/d lower maternal bw, lesser food consumption, and lower number of live foetuses. No effects at 250 mg/kg bw/d <u>Main study:</u> No effects in dams and offspring **NOAEL maternal/ developmental: 300 mg/kg bw/d**	
Pre-guideline Similar to OECD TG 414 GLP Subcutaneous administration of d-trans-tetramethrin 23 dams for examination at term (day 21) and 14 dams for examination of offspring at weaning Additional groups in which post natal development was examined	Rat Sprague Dawley 37 females per dose group	0, 100, 300, 1000 mg/kg bw/d Sub-cutaneous injection once daily on day 7-17 of gestation	<u>1000 mg/kg bw/d:</u> clonic convulsions, lower food consumption and decreases in bw, increases in liver and kidney weights of dams <u>300 mg/kg bw/d:</u> Lower food consumption No treatment-related effects in fetuses **NOAEL maternal: 300 mg/kg bw/d** **NOAEL developmental: 1000 mg/kg bw/d**	Satoh *et al*., 1982

Three independent developmental studies in rat showed that doses of 1000 mg tetramethrin/kg bw/d caused no effect on development. Another study in rat showed the same absence of developmental effect after administration of 1000 mg d-trans-tetramethrin/kg bw/d. Two independent studies in rabbit showed as doses of 90 and 500 mg tetramethrin/kg bw/d caused no effect on development. Another study in rabbit showed the same absence of developmental effect after administration of 300 mg d-trans-tetramethrin/kg bw/d.

A statistically non-significant increase in the incidence of rabbit foetal skeletal anomalies was noted at 500 mg tetramethrin/kg bw/d in one pre-guideline non-GLP study performed with Japanese White rabbits. It suggests that this rabbit strain might be more sensitive than New

Zealand White but RAC considered that the effects observed in this single study, compared with the other 7 independent studies reporting no effects, does not justify classification.

The one-generation reproduction toxicity study with tetramethrin reported lower numbers of corpora lutea and resulting implantations/live foetuses in F_1 and parental generations at 1000 mg/kg bw/d. However, according to the CLH report the incidence of this effect was only 10% and it is noted that it occurred only at the highest dose. In another pre-guideline one-generation study increase of stillborn pups and post-implantation loss (incidence not reported) at the same limit dose were also noted. In a two-generation reproduction toxicity study with d-trans-tetramethrin no alterations in fertility and sexual function were reported, although RAC noted that the highest dose used in this study was around 5 times lower than in the study with tetramethrin causing alterations.

In conclusion, no significant alterations in fertility and sexual function and in development could be seen and therefore, RAC agreed with the DS that **no classification for reproductive toxicity** (neither sexual function and fertility nor development) of d-trans-tetramethrin is warranted.

10.11 SPECIFIC TARGET ORGAN TOXICITY-SINGLE EXPOSURE

Table 57: Summary table of animal studies on STOT SE

Method Guideline, Deviation(s) from the guideline (if any)	Test substance, reference to table 5	Species Strain Sex no/group	Route of exposure	Dose levels duration of exposure	Results	Reference
OECD 403 Acute inhalation toxicity study with Tetramethrin in Wistar rats. GLP Technical deficiencies: Volume of air chamber 500 L, suggesting long time to achieve steady-state of concentration; no indication of whether concentration measurements performed in breathing zone; dense aerosol accumulation in chamber of G2 groups	Tetramethrin aerosol in cyclohexanone (50% w/v); mean aerosol particle size: G1: 0.67 ± 0.26 µm; G2: 0.68 ± 0.26 µm	Rat, Wistar Pre-study (G1): 2 M + 2 F Main study (G2): 5 M + 5 F	Head and nose exposure	0-5.63 ± 0.86 mg/L 4 h	LC_{50}: > 5.63 mg/L Slight lacrimation and nasal discharge on day 1 (all rats in G2), normal from day 2 onwards, otherwise no toxic signs observed. Absence of signs of acute toxicity in comparison to other studies suggests that concentration of 5.63 mg/L may not have been achieved in the breathing zone, study not appropriate for classification purposes	Venugopala RK, 2006, Toxicology Department, Advinus Therapeutics Private Limited., Endura Study N° 4414/05
Pre-guideline OECD 403, non-GLP. Acute study, inhalative, whole body 3 h exposure instead of 4 h in TG 403, 1981;	d-Tetramethrin dissolved in: deodorised kerosene mist, particle size	Rat, Sprague-Dawley 10 M + 10 F	Inhalative, whole body	0-0.026-0.131-0.243-0.595-1.18 mg/L (approx. 0-3.3-16.8-32.3-76.3-151 mg/kg bw);	LC_{50}: > 1.18 mg/L (151 mg/kg bw); ≥ 0.131 mg/L: Muscular fibrillation, urinary incontinence, limb paralysis, bradypnoe, irregular respiration (no. of affected animals and severity of findings not reported; toxic signs began	Suzuki T, Kohda H, Misaki Y, Okuno Y, Koyama Y, Miyamoto J, 1981, Sumitomo Report No. IT-10-0144 (Doc III

Method Guideline, Deviation(s) from the guideline (if any)	Test substance, reference to table 5	Species Strain Sex no/group	Route of exposure	Dose levels duration of exposure	Results	Reference
Dose-finding study for a subacute (28 day) study	1.23-1.51 µm			3 h	to appear 15 to 30 min, after initiation of exposure and disappeared 1 to 2 hours after exposure) at 1.18 mg/L: 1/10 females died	6.1.3)
Pre-guideline OECD 412 Non-GLP 28 day inhalation 3 h / day exposure	d-Tetra-methrin dissolved in: deodorised kerosene mist, particle size 1.23-1.51 µm	Rat, Sprague-Dawley 10 M + 10 F	Inhalative, whole body	0-0.026-0.049-0.087 mg/L 3 h / day exposure for 7 days aweek	≥ 0.087 mg/L: Slight bradypnea, irregular respiration, salivation directly after exposure (no. of affected animals and severity of findings not reported), no cumulative effect. Increase in leucocyte and decrease in eosinophils count at 0.087 mg/l NOAEL: 0.049 mg/L	Suzuki T, Kohda H, Misaki Y, Okuno Y, Koyama Y, Miyamoto J, 1981, Sumitomo Report No. IT-10-0144 (Doc III 6.1.3)
90 day inhalation OECD 413 GLP	Tetramethrin in corn oil Mist particles size 0.65 – 0.95 µm	Rat, Crj:CD(SD) 10 M + 10 F	Inhalative, whole body	0-0.020-0.134-0.824 mg/L (~0-4.5-29.8-183 mg/kg bw/d) 6 h/day for 5 days a week over 13-weeks	≥ 0.020 mg/L: increased liver and kidney weights (M/F) In addition at ≥ 0.134 mg/L (29.8 mg/kg bw/d): irregular respiration, bradypnea, decrease bw, changes of haematological (increased fibrinogen, prolongation of blood clotting activities) and blood chemistry parameters (increase in cholesterol and phospholipid levels (M) increased GGT and leucine aminotransferase levels (M)), dark-red discoloration of liver, soft and large liver, hepatocellular hypertrophy, hyaline droplets in renal tubules (M) In addition at 0.824 mg/L: decrease spontaneous activity. Nasal discharge, salivation, red tears, urinary incontinence.	Kawaguchi (1991) IT-10-0239 ((Doc III 6.4.3.(1)) Sumitomo

Method Guideline, Deviation(s) from the guideline (if any)	Test substance, reference to table 5	Species Strain Sex no/group	Route of exposure	Dose levels duration of exposure	Results	Reference
					Increased AlP, AST (M), and gamma-GT (M + F) levels, increased cholesterol levels (M + F), focal necroses in liver, hyaline casts in renal tubules (M) NOAEC: 0.02 mg/L (4.5 mg/kg bw/d) (for incidences and severity see Table 38d)	
90 day inhalation Determin-ation of the NOEL OECD 413 GLP	Tetra-methrin in corn oil Mist particles size 0.65 – 0.95 µm	Rat, Crj:CD(SD) 10 M + 10 F	Inhalative, whole body	0-0.002-0.004-0.02 mg/L (0-0.42-0.98-4.4 mg/kg bw/d) 6 h/day for 5 days a week over 13-weeks	Beside increase of liver weights in F F (absolute and relative), no adverse effects observed NOAEC: 0.02 mg/L (4.5 mg/kg bw/d)	Kawaguchi (1991 b) IT-10-0238 (Doc III 6.4.3.(2)) Sumitomo

Table 58: Summary table of human data on STOT SE

Type of data/report	Test substance, reference to table 5	Route of exposure	Relevant information about the study (as applicable)	Observations	Reference
No data					

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

Clinical signs of neurotoxicity were observed in the acute inhalation study performed by Suzuki et al. (1981; Sumitomo) with d-tetramethrin and have to be considered for classification as some of them were also observed in the 28-day study by Suzuki et al. (1981; Sumitomo) with d-tetramethrin as well as in the 90-day study performed by Kawaguchi (1991; Sumitomo) with tetramethrin. Significant neurotoxic effects (e.g. muscular fibrillation, urinary incontinence, limb paralysis, bradypnoe, irregular respiration) were observed in the acute and 90-day inhalative toxicity study with d-tetramethrin at concentrations of 0.131 mg/L or higher.

Slight bradypnea, irregular respiration and salivation directly after exposure were noted in the 28-day inhalative study (Suzuki et al., 1981) at ≥ 0.087 mg/L. A cumulative effect was not noted.

The observed effects can be presumed to have the potential to produce significant toxicity in humans on the basis of evidence from studies in experimental animals at a moderate concentration (in accordance with Guidance to Regulation (EC) No 1272/2008 on Classification, Labelling and Packaging of substances and mixtures; November 2013). Hence the proposed classification is "STOT SE 2; H371" for inhalative exposure.

According to guidance values in the above cited guidance document to Regulation (EC) No 1272/2008, classification as "STOT SE 1; H370" (inhalation rat: dust/mist/fume cat. 1: C ≤ 1.0 mg/L; Cat. 2: 5.0 ≥ C > 1.0) might be considered. However, since the severity of the effects was not clearly documented in the acute inhalation study (study was performed to estimate the LC_{50}), classification as "STOT SE 2; H371" is proposed. Although neurotoxic effects may have been responsible for the observed mortality resulting in the LC_{50} > 1.18 mg/L additional classification as "STOT SE 2; H371" is justified as the neurotoxic effects were already observed at approximately 10-fold lower concentrations. The severity of the effects cannot be estimated from the provided data.

10.11.2 Comparison with the CLP criteria

Table 59: Results of toxicity studies relevant for STOT SE in comparison to the CLP criteria

Toxicological results	**CLP criteria**	
Data on significant toxicity in humans are lacking. Since the severity of neurotoxic effects was not clearly documented in the acute inhalation study (study was performed to estimate the LC_{50}), classification as "STOT SE 1; H370" is not proposed.	Category 1 (H370) Oral (rat): C ≤ 300 mg/kg bw Dermal (rat or rabbit): C ≤ 1000 mg/kg bw Inhalative (rat, dust/mist/fume): ≤ 1 mg/L/4 h	Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following single exposure - reliable and good quality evidence from human cases or epidemiological studies; or - observations from appropriate studies in experimental animals in which significant and/or severe toxic effects of relevance to human health were produced at generally low exposure concentrations.
On the basis of evidence from studies in experimental animals at a moderate concentration (especially noted under specific target organ toxicity – repeated exposure), the observed neurotoxic effects were essentially acute effects and can be presumed to have the potential to produce significant toxicity in humans (in accordance with Guidance to Regulation (EC) No 1272/2008 on Classification, Labelling and Packaging of substances and mixtures; November 2013).	Category 2 (H371) Oral (rat): 2000 ≥ C > 300 mg/kg bw Dermal (rat or rabbit): 2000 ≥ C > 1000 mg/kg bw	Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following single exposure - observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at

Toxicological results	CLP criteria	
Hence the proposed classification is "STOT SE 2; H371" for inhalative exposure.	Inhalative (rat, dust/mist/fume): 5 ≥ C > 1 mg/L/4 h	generally moderate exposure concentrations.
	Category 3 (H335/H336) Guidance values do not apply (mainly based on human data)	Transient target organ effects This category only includes narcotic effects and respiratory tract irritation. These are target organ effects for which a substance does not meet the criteria to be classified in Categories 1 or 2 indicated above. These are effects which adversely alter human function for a short duration after exposure and from which humans may recover in a reasonable period without leaving significant alteration of structure or function.

10.11.3 Conclusion on classification and labelling for STOT SE

STOT SE 2; H371 (May cause damage to the central nervous system if inhaled).

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

Summary of the Dossier Submitter's proposal

The DS proposed classification of d-trans-tetramethrin as STOT SE 2 (H371; by inhalation route) on the basis of evidence from studies in experimental animals at a moderate concentration (especially noted under specific target organ toxicity-repeated exposure). The observed neurotoxic effects were essentially acute effects and can be presumed to have the potential to produce significant toxicity in humans.

Comments received during public consultation

One MSCA supported the classification proposal.

One MSCA questioned the proposed classification as STOT SE 2; H371 on the basis of the neurotoxicity seen in the acute inhalation study and 90-day repeated dose toxicity study because it is difficult to evaluate the significance and relevance of the observed effects due to

poor reporting in the studies. The MSCA requested further justification, especially in order to avoid double-classification for the neurotoxic effects through both STOT SE and Acute Tox.

The DS replied, sharing the concern for avoiding the double-classification, but stating that in the sub-acute inhalation toxicity the neurotoxicity was reported at 0.824 mg/L, every day only during the exposure period, and not after that. In addition, the effects were not cumulative and bradypnea, irregular respiration, decrease of spontaneous activity and salivation affected 100% of animals (both males and females) at this concentration (0.824 mg/L).

Assessment and comparison with the classification criteria

Clinical signs of neurotoxicity (muscular fibrillation, urinary incontinence, limb paralysis, bradypnea and irregular respiration) were observed in the acute inhalation study performed by Suzuki *et al*. (1981) with d-trans-tetramethrin at 0.131 mg/L and above. It is relevant that the toxic signs began to appear 15-30 minutes after initiation of the exposure and disappeared 1-2 hours after that. However, the severity of the effects was not clearly reported in this study.

In a dose-range finding study with d-trans-tetramethrin, muscular fibrillation, urinary incontinence, limb paralysis, bradypnea and irregular respiration were reported at exposure for 3 hours at concentrations above 0.131 mg/L (Suzuki *et al*., 1981). Toxic signs began to appear 15-30 minutes after initiation of exposure and disappeared 1-2 hours after exposure. However, the number of affected animals and severity of the findings were not reported.

The sub-acute (28-day) inhalation study with d-trans-tetramethrin reported slight bradypnea, irregular respiration and salivation after exposure (Suzuki *et al*., 1981) at concentrations equal or higher than 0.087 mg/L. A cumulative effect was not noted in this study.

The sub-chronic (90-day) inhalation study with tetramethrin reported, at doses of 0.134 mg/L, irregular respiration and decrease of spontaneous activity (Kawaguchi, 1991). In addition to these symptoms also bradypnea and salivation (100% incidence) and red tear and nasal discharge with 20 and 30% of incidence, respectively, were reported at 0.824 mg/L. It is also remarkable that most of the affected animals recovered after the exposure and that cumulative effects were not noted.

In the sub-chronic oral study in rat, at 151 mg/kg bw/d, a neurotoxic effect consisting of significant increase in landing foot splay in males was reported, but no further information about severity and incidence could be found. In the 6-month oral study in dogs also increased nervous tremors at 90 mg/kg bw/d was observed, but this effect was not reported at 180 mg/kg bw/d.

RAC notes that d-trans-tetramethrin belongs to the family of pyrethroid biocides and it is well known that these compounds exert their neurotoxicity mainly through impairments in the performance of ionic channels found in the plasmatic membranes of neurons (Lund and Narahashi, 1982). These channels are also found in mammals and therefore humans are also potential targets for the neurotoxicity of pyrethroids. In rats, acute poisoning syndrome associated with type I pyrethroids (the sub-family of d-trans-tetramethrin) is characterised by neurological effects such as aggressive sparring, whole body tremor and prostration

(Verschoyle and Aldridge, 1980). Symptoms of neurotoxicity were observed for both tetramethrin and d-trans-tetramethrin after inhalation exposure as irregular respiration, bradypnea and decreased spontaneous activity; whereas tremor, urinary incontinence and limb paralysis were only observed in the inhalation study with d-trans-tetramethrin.

RAC notes that the neurotoxicity in the repeated toxicity studies was not cumulative and mostly disappeared after the exposure ended and therefore RAC considers that these neurotoxic effect are indeed acute effects that appeared after each exposure, hence justifying classification as STOT SE. At the same time, the lack of consistency in the neurotoxicity reported in the repeated oral studies induces RAC to discard this route of exposure for STOT SE classification and therefore RAC proposes inhalation as the only relevant route for classification as STOT SE.

The CLP Regulation establishes that substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following single exposure at concentrations lower than 1 mg/L should be classified as STOT SE Category 1. RAC notes that all three available studies show neurotoxicity effects below this concentration. However, RAC also notes that the neurotoxicity was not always clearly documented and classification of d-trans-tetramethrin as Category 1 is thus not proposed.

STOT SE Category 3 should cover "transient" respiratory tract irritation and narcotic effects occurring after single exposure. RAC notes that such effects were not reported and therefore the classification of d-trans-tetramethrin as STOT SE Category 3 is not supported.

The CLP Regulation establishes that substances that can be presumed to have the potential to be harmful to human health following single exposure at concentrations ranging between 1 and 5 mg/L should be classified as STOT SE Category 2. The available information shows neurotoxicity after single exposure at concentrations below 1 mg/L but due to the deficiencies in the reporting regarding severity and incidence of the effects, Category 2 is considered more appropriate than Category 1.

In conclusion, RAC agreed with the DS's proposal for classification of d-trans-tetramethrin as **STOT SE Category 2 (H371: May cause damage to nervous system by inhalation route).**

10.12 SPECIFIC TARGET ORGAN TOXICITY-REPEATED EXPOSURE

Table 60: Summary table of animal studies on STOT RE

Method Guideline, Deviation(s) from the guideline (if any)	Test substance, reference to table 5	Species Strain Sex no/group	Route of exposure	Dose levels duration of exposure	Results	Reference
Sim. to OECD 407, non-GLP	d-Tetra-methrin	Rat, CRJ:CD (SD), 10 M + 10 F / dose group	Oral, dietary,	0-300-3000-10000 ppm (0-30-290/295-965/940 mg/kg bw/d (M/F)) 28 days	From 3000 ppm onwards: Increased cholesterol, albumin, total protein, and glucose levels , increased liver weights (regarded as adaptive) 10000 ppm: increased ALT and AST levels; Liver: Focal necrosis, (M), enlargement (M + F), peripheral lobular hypertrophy of parenchymal cells Haematology: Increased thrombocyte levels (M), decreased haematocrit and haemoglobin (M) Other: Increased urinary protein levels (M), de-creased bw gain NOAEL: 3000 ppm (290/295 mg/kg bw/d (M/F))	Hosokawa, S. (1985) Sumitomo report No. IT-20-0188 (Su: Doc III-A6.3.1)
OECD 408 GLP	Tetra-methrin in food	Rat, Wistar 10 M+10 F /group	Oral, dietary, Including 4-week recovery period	0-500-1000-2000 ppm (0-38-76-151 mg/kg bw/d), Recovery group at 0 + 2000 ppm 90 days	1000 + 2000 ppm: Increased liver weights (rel./abs; M) and cholesterol (M/F), hypertrophy of hepatocytes (M) 2000 ppm: Neurological effects: significant increase in landing foot splay (M) increased liver weight (F) NOAEL: 1000 ppm (76 mg/kg bw/d)	Malleshappa, H.N. (2002). Endura Study N°3343/01, 24 (En: Doc III-A6.4.1.1)
Sim. to OECD 408, non-GLP	d-Tetra-methrin in corn oil	Rat, Sprague-Dawley, 3 mo: 12 M + 12 F/dose	Oral, dietary	0-100-300-1000-3000 ppm (0-5.8/7-17/21-58/71-	1000 ppm onwards: Slightly increased urinary protein and cholesterol levels (M/F), increased liver (M/F) and kidney weights (M)	Hosokawa S, Hiromori T, Seki T, Okuno Y, Miyamoto J, 1980,

Method Guideline, Deviation(s) from the guideline (if any)	Test substance, reference to table 5	Species Strain Sex no/group	Route of exposure	Dose levels duration of exposure	Results	Reference
		group (satellite groups) 6 mo: 20 M + 20 F / dose group (main groups)		178/214 mg/kg bw/d (M/F)) 3 months + 6 months	In addition at 3000 ppm: Increased kidney weights (MF), significant reduced bw (M/F) , swelling and 'luster surface' <u>of the liver</u> (M), eosinophilic bodies in renal tubular epithelium (M) NOAEL: 1000 ppm (58/71 mg/kg bw/d (M+F))	Sumitomo Report No. IT-00-0139 (Su: Doc III-A6.4.1)
Sim. to OECD 408, non-GLP	Tetra-methrin	Rat, Sprague-Dawley, 16 M + 16 F/dose group	Oral, dietary	0-500-1500-5000 ppm (0-30-95-325 mg/kg bw/d) 6 months	<u>5000 ppm:</u> Increased cholesterol levels (M/F), increase in absolute (M) and relative (M/F) liver weight, decreased haemoglobin levels (M), decreased AlP and ALAT (M), decreased body weight gain (M+F) NOAEL 1500 ppm (95 mg/kg bw/d)	Suzuki, T, Okuno Y., 1977, Sumitomo Report No. IT-60-0015 (Su: Doc III-A6.4.1(2))

Method Guideline, Deviation(s) from the guideline (if any)	Test substance, reference to table 5	Species Strain Sex no/group	Route of exposure	Dose levels duration of exposure	Results	Reference
Sim. to OECD 409, non-GLP	Tetra-methrin	Dog, Beagle, 6 M + 6 F / dose group	Oral, dietary	0-1250-2500-5000 ppm (0-45-90-180 mg/kg bw/d) 6 months	<u>2500 ppm onwards:</u> Decrease in albumin/globulin ratio (M/F), increase in cholesterol (F) increased liver weight (M), increased nervousness, tremors (M+F) <u>5000 ppm:</u> Decrease in total protein (M/F), albumin (M/F); increase in cholesterol (M/F); decrease blood urea nitrogen (M), decreased haematocrit and erythrocyte counts (M); relative liver weight increase (M/F); decrease in absolute + relative ovary weight; Ovary, mammary gland: absence of histopathological changes associated with oestrus (i.e. corpora lutea; endometrial hypertrophy; mammary gland hyperplasia, secretary activity, and stromal and ductal proliferation) NOAEL: 2500 ppm (90 mg/kg bw/d)	Pence D, 1981, Sumitomo Report No. IT-11-0098 (Su: Doc III-A6.4.1(1))
Sim. to OECD 452 GLP	Tetra-methrin	Dog, Beagle, 4 M + 4 F/dose group	Oral, dietary	0-300-1200-5000-10000 ppm (0-8/9-36/36-147/157-286/325 mg/kg bw/d (M/F)) 1 year	1200 ppm: Slightly increased cholesterol and phospholipids (M), increased liver weight (M) <u>≥ 5000 ppm:</u> Lower bw (F), increased cholesterol, phospholipid and AlP levels (M+F), decreased albumin levels (F), decreased erythrocyte count, haemoglobin and haematocrit levels (F), increased liver weight (M) NOAEL: 1200 ppm (36 mg/kg bw/d) Slight changes in liver physiology and clinical chemistry were already	Walker M D, 1996, Sumitomo Report No. IT-0276 (Doc. III-A6.5.4)

Method Guideline, Deviation(s) from the guideline (if any)	Test substance, reference to table 5	Species Strain Sex no/group	Route of exposure	Dose levels duration of exposure	Results	Reference
					observed at 1200 ppm in males. However, the deduced NO(A)EL is based on aggravation of liver effects (increase in liver weight also in females, increase in AlP) and on occurrence of haematological effects (increased platelet count).	
90 day inhalation OECD 413 GLP	Tetra-methrin in corn oil Mist particles size 0.65 – 0.95 µm	Rat, Crj:CD(SD) 10 M + 10 F	Inhalative, whole body	0-0.020-0.134-0.824 mg/L (~0-4.5-29.8-183 mg/kg bw/d) 6 h/day for 5 days a week over 13-weeks	≥ 0.020 mg/L: increased liver and kidney weights (M/F) In addition at ≥ 0.134 mg/L (29.8 mg/kg bw/d): irregular respiration, bradypnea, decrease bw, changes of haematological (increased bilirubin and urobilinogen) and blood chemistry parameters (increased cholesterol and phospholipid levels (M) increased GGT and leucine aminotransferase levels (M)), dark-red discoloration of liver, soft and large liver, hepatocellular hypertrophy, hyaline droplets in renal tubules (M) In addition at 0.824 mg/L: decrease spontaneous activity. Nasal discharge, salivation, red tears, urinary incontinence. Increased AlP, AST (M), and gamma-GT (M + F) levels, increased cholesterol levels (M + F), focal necroses in liver, hyaline casts in renal tubules (M) NOAEC: 0.02 mg/L (4.5 mg/kg bw/d)	Kawaguchi (1991 a) IT-10-0239 (Doc. III 6.4.3.1) Sumitomo

Table 61: Summary table of human data on STOT RE

Type of data/report	Test substance, reference to table 5	Route of exposure	Relevant information about the study (as applicable)	Observations	Reference
No Data					

10.13 SHORT SUMMARY AND OVERALL RELEVANCE OF THE PROVIDED INFORMATION ON SPECIFIC TARGET ORGAN TOXICITY – REPEATED EXPOSURE

Toxicity of orally administered substance was tested in rats over 4 weeks in a study with d-tetramethrin (Hosokawa, 1985; Sumitomo), in a 90-day study with tetramethrin (Malleshappa, 2002; Endura) as well as in one 3-6-month study in rats with d-tetramethrin (Hosokawa, 1980; Sumitomo) and in a study over 6 months with tetramethrin (Suzuki et al., 1977; Sumitomo). Common effects observed in these studies included a decrease in body weight gain, haematological alterations, changes in clinical chemistry, increased liver weight and dose-dependent liver toxicity. The LOAELs were predominantly based on liver toxicity (gross and histopathology and clinical chemistry) and haematological findings. The derived subacute and subchronic NOAELs were 290 mg/kg bw/d for exposure over 4 weeks (d-tetramethrin; Hosokawa 1985, Sumitomo), 90 mg/kg bw/d (2500 ppm) and 36 mg/kg bw/d (1200 ppm) for exposure of Beagle dogs to tetramethrin over one year (Walker 1986; Sumitomo) and 6 months (Pence, 1981, Sumitomo).

Changes in blood chemistry (decreased albumin/globulin ratio, decrease in total protein and albumin, increased cholesterol levels) and haematological parameters (decreased blood urea nitrogen decreased haematocrit and erythrocyte counts), increased liver weight as well as increased nervousness and tremors were noted at 36 mg/kg bw/d (Walker, 1996) and 90 mg/kg bw/d (Pence 1981). In the study conducted by Pence (1981) female dogs showed a decrease in absolute + relative ovary weight as well as absence of histopathological changes associated with oestrus (i.e. corpora lutea; endometrial hypertrophy; mammary gland hyperplasia, secretary activity, and stromal and ductal proliferation) at the highest dose of 5000 ppm (180 mg/kg bw/d). This finding was not reproducible in the study performed by Walker (1996).

Increased AlP levels, increased liver weights, and decreased erythrocyte count, haemoglobin and hematocrit levels, were observed from dose level of 5000 ppm (147/157 mg/kg bw/d; M/F) onwards.

These values were supported by NOAELs of 76 mg/kg bw (1000 ppm; Malleshappa 2002; Endura) and 95 mg/kg bw (1500 ppm; Suzuki and Okuno 1977; Sumitomo) in subchronic toxicity studies between 3 and 6 months in rats.

Furthermore, two overlapping studies were performed to test subchronic (3 months) inhalative toxicity of tetramethrin in Sprague Dawley Crj:CD rats (Kawaguchi, 1991a and b; Sumitomo), the study IT-10-238 (Kawaguchi 1991 b) displaying a refined spacing of the low concentration range as compared to the study IT-10-239 (Kawaguchi 1991 a). In the study No. -238 no adverse effects were observed up to a concentration of 0.02 mg tetramethrin /L air (4.4 mg/kg bw/d). In the study -0239

(Kawaguchi 1991 a), adverse effects relevant for the LOAEC of 0.134 mg/L comprised lower body weight gain (males, mid and high dose; females high dose without statistical significance), changes in clinical chemistry (higher total protein, lower albumin, higher alpha2-globulin resulting in lower A/G ratio in both sexes of the high dose group, higher cholesterol, phospholipid and Γ-Glutamyl transpeptidase in males of the mid and high dose and in females of the high dose),, urinalysis (significantly increased blirubin and urobilinogen in both sexes, mid and high dose), haematology (prolonged prothrombin time, higher activated partial thromboplastin time (APTT) and fibrinogen in both sexes, high dose) organ toxicity (increased absolute and relative liver weights in both sexes from the low dose onwards), macro- (dark red liver, soft and large liver in males of the mid and high dose, with lesser extent also in females of these dose groups), and histolopathological organ abnormalities (In the liver: focal necrosis and bile duct hyperplasia in males of the high dose group, massive necrosis in 1/10 female of the high dose group, hepatocellular hypertrophy in both sexes from the mid and high dose group. In the kidney: Basophilic tubules, eosinophilic bodies, hyaline casts and hyaline droplets in tubules with higher incidence and/or severity grading in males of the mid and high dose). Acute clinical signs occurred during the daily exposure period (irregular respiration, bradypnoe). Both 3-month studies pointed to a NOAEC of 0.02 mg/L (approximate daily dose of 4.5 mg/kg bw) and a LOAEC of 0.134 mg/L. (29.8 mg/kg bw/d). Conversion of inhaled concentrations into inhaled daily doses was based on default assumptions regarding body weight, inhalation volume and 100 % availability and suggests a significantly higher sensitivity to d-tetramethrin and tetramethrin by the inhalative than the oral route of administration in the rat (NOAEL for tetramethrin of 4.5 mg/kg bw/d, 3 months inhalative versus 95 mg/kg bw/d, in subchronic oral study in rats (6 months; Suzuki et al. 1977; Sumitomo).

The effects in the 3-month inhalation toxicity study (Kawaguchi 1991; see table 38d) were more pronounced in male rats of the mid and high dose groups than in female rats of these dose groups and were considered adverse. Anyhow, in comparison with the criteria for specific target organ toxicity after repeated exposure, on the basis of the weight of evidence and by the use of expert judgement the observed changes in liver physiology and clinical chemistry were considered not adequate for a classification of tetramethrin as specific target organ toxicants following repeated exposure (STOT RE).

The observed neurotoxic effects seen in the 28-day and 90-day studies were essentially acute effects and they were considered relevant for classification as specific target organ toxicant – single exposure (STOT SE) (for neurotoxicity). In the 90-d rat study with inhalative exposure (Kawaguchi et al. 1991a) concentration dependent incidences of irregular respiration were observed in mid- and high concentration groups (LOAEC: 0.134 mg/L) over the entire period of exposure. Bradypnoea and decrease of spontaneous activity occurred in a concentration dependent manner and were considered adverse in the highest exposure group (animals affected per exposure day, days with affected animals). Bradypnoea was observed up to day 12 (males) resp. day 45 (females), decrease of spontaneous activity up to day 45 of exposure (males and females). The results are summarised in Table 38d. The severity of effects was not reported.

Table 62: Summary table of selected adverse effects in the 3-month inhalative toxicity study in rats (Kawaguchi; 1991a)

Males	**Dose groups**			
Effect	**O mg/L (Vehicle control)**	**0.02 mg/L (4.5 mg/kg bw/D)**	**0.134 mg/L 30 mg/kg bw/d)**	**0.824 mg/L 183 mg/kg bw/d)**
Mean body weight on day 89 ± SD (g)	552 ± 52.5	550 ± 62.4	494 * ± 31.8	454 ** ± 43.4

Males	**Dose groups**			
Effect	**O mg/L (Vehicle control)**	**0.02 mg/L (4.5 mg/kg bw/D)**	**0.134 mg/L 30 mg/kg bw/d)**	**0.824 mg/L 183 mg/kg bw/d)**
Final body weight (g)	524 ± 51.2	521 ± 61.9	470* ± 28.4	422** ± 39.9
Prothrombin time (sec)	17.7 ± 1.64	17.3 ± 1.95	18.9 ± 2.94	21.3** ± 3.35
Activated partial thromboplastin time (sec)	24.0 ± 1.62	23.3 ± 2.93	24.9 ± 2.37	27.3** ± 2.79
Fibrinogen (mg/dl)	233.9 ± 14.85	234.9 ± 15.48	235.2 ± 16.77	261.2** ± 22.75
Total protein (g/dl)	6.0 ± 0.25	6.2* ± 0.28	6.2* ± 0.24	6.5** ± 0.25
Albumin (%)	54.0 ± 1.54	51.5* ± 0.23	52.0 ± 1.93	50.0** ± 3.46
Ά2- Globulin (%)	4.8 ± 0.43	5.2 ± 0.47	5.4* ± 0.58	5.9** ± 0.96
A/G ratio	1.18 ± 0.075	1.07 ± 0.117	1.09 ± 0.085	1.05* ± 0.150
Cholesterol (mg/dl)	70 ± 18.8	72 ± 14.9	92* ± 20.3	114** ± 29.9
Phospholipid (mg/dl)	105 ± 21.8	110 ± 21.0	135* ± 29.5	172** ± 44.6
Γ-Glutamyl transpeptidase (U/l)	1 ± 1.2	1 ± 1.2	4** ± 2.1	13** ± 3.6
Liver weight (abs., g)	14.20 ± 1.945	15.58 ± 2.532	16.00* ± 1.480	17.68** ± 2.212
Liver weight (rel., g%)	2.71 ± 0.156	2.98* ± 0.217	3.42** ± 0.194	4.19** ± 0.369
Gross pathological changes				
Liver: Dark red	0/10	0/10	4/10	9/10
Liver: Soft	0/10	0/10	3/10	3/10
Liver: Large	0/10	0/10	4/10	6/10
Histopathological examination				
Liver: Focal necrosis	1/10	0/10	0/10	3/10
Liver: Hepatocelluar hypertrophy	0/10	0/10	5/10 (slight)	5/10 (slight) 4/10 (mild)
Liver: Bile duct hyperplasia	0/10	0/10	0/10	4/10 (slight)
Kidney: hyaline droplets in tubules	2/10 (slight)	4/10 (slight)	3/10 (slight) 4/10(mild) 1/10 (severe)	2/10 (slight) 3/10(mild) 5/10 (severe)
Clinical signs in first 2 weeks (10 days of exposure)				
Bradypnoea **days** with ≥ 1 animal affected/days with exposure (mean **animals** affected per day)	1/10 (0)	0/10 (0)	0/10 (0)	10/10 (6)

Exposure: days 1 - 14§				
Irregular respiration: # days with ≥ 1 animal affected/days with exposure (mean animals affected per day) Exposure: days 1 - 14§§	7/10 (2)	9/10 (2)	10/10 (7)	10/10 (10)
Decrease of spontaneous activity # days with ≥ 1 animal affected/days with exposure (mean animals affected per day) Exposure: days 1 - 14§§§	1/10 (0)	1/10 (0)	8/10 (2)	10/10 (9)

§: days 6, 7, 13, 14: non-exposure, bradypnoea occurred only incidental after day 12 of exposure
§§: days 6,7, 13, 14: non-exposure, irregular respiration: all animals in the two highest exposure groups affected on every exposure day during entire treatment period, groups vehicle control and lowest concentration: incidental following approx. 2 wks of exposure
§§§: days 6, 7, 13, 14: non-exposure, decrease in spontaneous activity, decreases in spontaneous activity observed at highest concentration up to day 45 of exposure, afterwards no more incidences reported

Females	**Dose groups**			
Effect	**O mg/L**	**0.02 mg/L (4.5 mg/kg bw/D)**	**0.134 mg/L 30 mg/kg bw/d)**	**0.824 mg/L 183 mg/kg bw/d)**
Mean body weight on day 89 ± SD (g)	310 ± 30.2	305 ± 27.7	296 ± 24.3	288 ± 22.8
Final body weight (g)	291 ± 27.9	287 ± 27.1	274 ± 22.7	259 ± 21.6
Prothrombin time (sec)	14.0 ± 0.25	13.9 ± 0.23	14.1 ± 0.25	14.5** ± 0.76
Activated partial thromboplastin time (sec)	20.7 ±1.46	20.2 ± 1.43	20.6 ± 1.50	22.4** ± 1.59
Fibrinogen (mg/dl)	173.7 ± 17.32	161.1 ± 18.44	163.8 ± 13.49	190.8* ± 16.86
Total protein (g/dl)	6.5 ± 0.35	6.5 ± 0.45	6.6 ± 0.28	7.1** ± 0.20
Albumin (%)	59.3 ± 3.49	50.4 ± 2.26	58.3 ± 3.07	53.0** ± 2.39
Ά2- Globulin (%)	5.0 ± 0.77	5.5 ± 0.48	5.3 ± 0.41	6.1 ± 0.78
A/G ratio	1.47 ± 0.204	1.47 ± 0.139	1.41 ± 0.183	1.16** ± 0.109
Cholesterol (mg/dl)	80 ± 10.1	83 ± 14.1	91 ± 17.9	117** ± 17.8
Phospholipid (mg/dl)	148 ± 21.5	158 ± 35.1	162 ± 23.4	196 ± 25.4
Γ-Glutamyl transpeptidase (U/l)	1 ± 1.2	1 ± 1.2	2 ± 1.1	9** ± 7.5
Liver weight (abs., g)	7.48 ± 0.885	7.96 ± 1.341	8.78** ± 0.811	10.24** ± 0.639
Liver weight (rel., g%)	2.57 ± 0.135	2.77* ± 0.288	3.21** ± 0.071	3.97** ± 0.273
Gross pathological changes				
Liver: Dark red	0/10	0/10	1/10	7/10
Liver: Soft	0/10	0/10	0/10	1/10
Liver: Large	0/10	0/10	2/10	7/10
Histopathological examination				
Liver: Massive necrosis	0/10	0/10	0/10	1/10
Liver: Hepatocelluar hypertrophy	0/10	0/10	2/10 (slight)	9/10 (slight)
Liver: Bile duct hyperplasia	0/10	0/10	0/10	1/10 (slight)
Clinical signs in first 2 weeks (10 days of exposure)				
Bradypnoea **# days** with ≥ 1 animal affected/days with exposure (mean **animals** affected per day) Exposure: days 1 - 14§	0/10 (0)	0/10 (0)	1/10 (2)	10/10 (5)
Irregular respiration: **# days** with ≥ 1 animal affected/days with exposure (mean **animals** affected per day) Exposure: days 1 - 14§§	7/10 (1)	6/10 (1)	10/10 (3)	10/10 (10)
Decrease of spontaneous activity **# days** with ≥ 1	0/10 (0)	0/10 (0)	3/10 (0)	10/10 (6)

animal affected/days with exposure (mean **animals** affected per day) Exposure: days 1 - 14§§§				

§: days 6, 7, 13, 14: non-exposure, bradypnoea occurred only incidental after day 12 of exposure
§§: days 6,7, 13, 14: non-exposure, irregular respiration: all animals in the two highest exposure groups affected on every exposure day during entire treatment period, groups vehicle control and lowest concentration: incidental following approx. 2 wks of exposure
§§§: days 6, 7, 13, 14: non-exposure, decrease in spontaneous activity, decreases in spontaneous activity observed at highest concentration up to day 45 of exposure, afterwards no more incidences reported
** Significantly different from vehicle control (P<0.01)
* Significantly different from vehicle control (P<0.05)

Table 63: Correction of the effective dose

Study reference	**Effective dose (mg/kg/d)**	**Length of exposure**	**Correction of the effective dose when extrapolated to 90-day exposure**	**Classification supported by the study**
Not necessary				

10.13.1 Comparison with the CLP criteria

Table 64: Results of toxicity studies relevant for STOT RE in comparison to the CLP criteria

Toxicological results	CLP criteria
Data on significant toxicity in humans are lacking and guidance values are not applicable.124	Category 1 (H372): Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations. Equivalent guidance values for 28-day and 90-day studies: Inhalation dust/mist/fume, rat: 28-day: ≤ 0.06 mg/L/6 h/d 90-day: ≤ 0.02 mg/L/6 h/d
On the basis of evidence from studies with repeated exposure in experimental animals at a moderate concentration, the observed effects (liver) can be presumed to represent adaptative effects only and not to have the potential to produce significant toxicity in humans (in accordance with Guidance to Regulation (EC) No 1272/2008 on Classification, Labelling and Packaging of substances and mixtures; November 2013). Hence, no classification for “STOT-RE” for oral exposure is proposed.	Category 2 (H373): Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure. Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. Guidance dose/concentration values are provided below (see 3.9.2.9) in order to help in classification. In exceptional cases human evidence can also be used to place a substance in Category 2. Equivalent guidance values for 28-day and 90-day studies: Inhalation dust/mist/fume, rat: 28-day: ≤ 0.6 mg/L/6 h/d 90-day: ≤ 0.2 mg/L/6 h/d

10.13.2 Conclusion on classification and labelling for STOT RE

Classification and labelling for STOT RE is not proposed.

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

Summary of the Dossier Submitter's proposal

The DS proposed no classification for STOT RE because they considered that, based on evidence from studies with repeated exposure in experimental animals at a moderate concentration, the observed effects in liver can be presumed to represent adaptive effects only and not to have the potential to produce significant toxicity in humans.

Comments received during public consultation

One MSCA supported the no classification proposal.

Additional key elements

The table below summarises the main relevant findings in the repeated toxicity studies with tetramethrin.

Table: Summary table of the animal repeated dose toxicity studies with tetramethrin.

Method	Species Strain Sex N°/group Route Dose level Exposure duration	Results	Reference
OECD TG 408 GLP	Rat Wistar 10 M + 10 F per dose group Oral (dietary) 0, 500, 1000, 2000 ppm (0, 38, 76, 151 mg/kg bw/d) 90 days (including a 4-week recovery period)	1000 + 2000 ppm: Increased liver weights (rel./abs.; M) and cholesterol (M/F), hypertrophy of hepatocytes (M) 2000 ppm: *Neurological effects*: significant increase in landing foot splay (M) increased liver weight (F) **NOAEL: 1000 ppm**	Malleshappa, 2002
Similar to OECD TG 408 Non-GLP	Rat Sprague-Dawley	5000 ppm: Increased cholesterol levels (M/F), increase in absolute (M) and relative (M/F) liver weight, decreased haemoglobin levels (M), decreased ALP	Suzuki and Okuno, 1977

	16 M + 16 F per dose group Oral (dietary) 0, 500, 1500, 5000 ppm (0, 30, 95, 325 mg/kg bw/d) 6 months	and ALAT (M), decreased body weight gain (M+F) **NOAEL: 1500 ppm**	
Similar to OECD TG 409 Non-GLP	Dog Beagle 6 M + 6 F per dose group Oral (dietary) 0, 1250, 2500, 5000 ppm (0, 45, 90, 180 mg/kg bw/d) 6 months	≥ 2500 ppm: Decrease in albumin/globulin ratio (M/F), increase in cholesterol (F) increased liver weight (M), increased nervousness, tremors (M+F) 5000 ppm: Decrease in total protein (M/F), albumin (M/F); increase in cholesterol (M/F); decrease blood urea nitrogen (M), decreased haematocrit and erythrocyte counts (M); relative liver weight increase (M/F); decrease in absolute + relative ovary weight *Ovary, mammary gland*: no histopathological changes associated with oestrus **NOAEL: 2500 ppm**	Pence, 1981
Similar to OECD TG 452 GLP	Dog Beagle 4 M + 4 F per dose group Oral (dietary) 0, 300, 1200, 5000, 10000 ppm (0, 8/9, 36/36, 147/157, 286/325 mg/kg bw/d (M/F)) 1 year	1200 ppm: Slightly increased cholesterol and phospholipids (M), increased liver weight (M) ≥ 5000 ppm: Lower bw (F), increased cholesterol, phospholipid and ALP levels (M+F), decreased albumin levels (F), decreased erythrocyte count, haemoglobin and haematocrit levels (F), increased liver weight (M) **NOAEL: 1200 ppm**	Walker, 1996
OECD TG 413 GLP	Rat Crj:CD(SD) 10 M + 10 F per dose group Inhalative (whole body)	≥ 0.020 mg/L: Increased liver and kidney weights (M/F) ≥ 0.134 mg/L: Irregular respiration, bradypnea, decreased bw, changes in clinical chemistry (increased bilirubin and urobilinogen, increased cholesterol and phospholipid levels (M), increased GGT and leucine aminotransferase levels	Kawaguchi, 1991

	0, 0.020, 0.134, 0.824 mg/L 6 h/day for 5 days a week over 13 weeks Mist particles size: 0.65-0.95 µm	(M)), dark-red discoloration of liver, soft and large liver, hepatocellular hypertrophy, hyaline droplets in renal tubules (M) <u>0.824 mg/L:</u> Decreased spontaneous activity, nasal discharge, salivation, red tears, urinary incontinence. Increased ALP, AST (M), and GGT (M+F) levels, increased cholesterol levels (M+F), focal necrosis in liver, hyaline casts in renal tubules (M) **NOAEC: 0.02 mg/L**	

Assessment and comparison with the classification criteria

The table below summarises the main relevant findings in the repeated toxicity studies with <u>d-trans-tetramethrin</u>.

Table: Summary table of the animal repeated dose toxicity studies with d-trans-tetramethrin.

Method	Species Strain Sex Nº/group Route Dose levels Exposure duration	Results	Reference
Similar to OECD TG 407 Non-GLP	Rat CRJ:CD (SD) 10 M + 10 F per dose group Oral (dietary) 0, 300, 3000, 10000 ppm (0, 30, 290/295, 965/940mg/kg bw/d (M/F)) 28 days	<u>≥ 3000 ppm:</u> Increased cholesterol, albumin, total protein, and glucose levels, increased liver weights <u>10000 ppm:</u> *Haematology and clinical chemistry*: increased ALT and AST levels, increased thrombocyte levels (M), decreased haematocrit and haemoglobin (M) *Liver*: Focal necrosis, (M), enlargement (M/F), peripheral lobular hypertrophy of parenchymal cells *Other*: Increased urinary protein levels (M), decreased bw gain **NOAEL: 3000 ppm**	Hosokawa, 1985
Similar to OECD TG 408 Non-GLP	Rat Sprague-Dawley 3 month: 12 M	<u>≥ 1000 ppm:</u> Slightly increased urinary protein and cholesterol levels (M/F), increased liver (M/F) and kidney weights (M)	Hosokawa *et al.*, 1980

	+ 12 F per dose group 6 month: 20 M + 20 F per dose group Oral (dietary) 0, 100, 300, 1000, 3000 ppm (0, 5.8/7, 17/21, 58/71, 178/214 mg/kg bw/d (M/F)) 3 months + 6 months	3000 ppm: Increased kidney weights (M/F), significant reduced bw (M/F) , swelling and 'luster surface' of the liver (M), eosinophilic bodies in renal tubular epithelium (M) **NOAEL: 1000 ppm**	

The table below gives an overview of adverse effects relevant for STOT RE classification that were consistently observed in the available repeated dose toxicity studies.

Table: Adverse effects of tetramethrin and d-trans-tetramethrin relevant for STOT RE classification.

Effect	Study	Lowest reported dose (mg/kg bw/d; except in inhalation studies)	Guidance value for STOT RE classification (mg/kg bw/d; except in inhalation studies)
TETRAMETHRIN			
Mortality	Rabbit, teratogenicity	500	50 ≤ C ≤ 500
Neurotoxicity	Rat, 90 days, oral Dog, 6 months, oral Rat, 90 days, inhalation	151 90 0.824 mg/L/6 h/d	10 ≤ C ≤ 100 5 ≤ C ≤ 50 0.02 ≤ C ≤ 0.2 mg/L/6 h/d
Haematological and clinical chemistry changes	Rat, 90 days, oral Rat, 6 months, oral Dog, 6 months, oral Dog, 1 year, oral **Rat, 90 days, inhalation**	176 325 90 36 **0.134 mg/L/6 h/d**	10 ≤ C ≤ 100 5 ≤ C ≤ 50 5 ≤ C ≤ 50 2.5 ≤ C ≤ 25 **0.02 ≤ C ≤ 0.2 mg/L/6 h/d**
Nephrotoxicity	**Rat (90 days, inhalation)** Teratogenicity, rat	**0.134 mg/L/6 h/d** 1000	**0.02 ≤ C ≤ 0.2 mg/L/6 h/d** 90 ≤ C ≤ 900
Hepatotoxicity	**Rat, 90 days, oral** Rat, 6 months, oral Dog, 6 months, oral Dog, 1 year, oral **Rat, 90 days, inhalation** Rabbit, teratogenicity Rat, teratogenicity Rat, carcinogenicity	**76** 325 90 36 **0.134 mg/L/6 h/day** 500 1000 125 (M)/165 (F)	**10 ≤ C ≤ 100** 5 ≤ C ≤ 50 5 ≤ C ≤ 50 2.5 ≤ C ≤ 25 **0.02 ≤ C ≤ 0.2 mg/L/6 h/d** 50 ≤ C ≤ 500 90 ≤ C ≤ 900 1.25 ≤ C ≤ 12.5
d-trans-tetramethrin			
Mortality	Rabbit, teratogenicity	1000	50 ≤ C ≤ 500

Haematological and clinical chemistry changes	**Rat, 28 days, oral**	**290 (M)/295 (F)**	**30 ≤ C ≤ 300**
Nephrotoxicity	Rat, 6 month, oral Rat, teratogenicity	58 (M)/71 (F) 1000	5 ≤ C ≤ 50 90 ≤ C ≤ 900
Hepatotoxicity	Rat, 28 days, oral Rat, 6 months, oral Rat, teratogenicity	965 (M)/940 (F) 178 (M)/214 (F) 1000	30 ≤ C ≤ 300 5 ≤ C ≤ 50 90 ≤ C ≤ 900

Data were taken from the studies summarised in this section plus the reproductive toxicity and carcinogenicity studies described in next sections. Bold text refers to effects appearing at doses relevant for classification as STOT RE.

Lethality was reported in several range-finding developmental toxicity studies, always at doses of 500 mg tetramethrin/kg bw/d or 1000 mg d-trans-tetramethrin/kg bw/d. These mortalities appeared at doses well above the limit doses to be considered for STOT RE classification and therefore RAC considers this effect not relevant for classification.

Neurotoxicity was reported in the 90-day toxicity studies (both by oral and inhalation route) in rats and in the 6-month toxicity study in dogs. These effects were essentially acute effects that were already considered for STOT SE classification and, in addition, appeared above the limits for warranting classification as STOT RE. Therefore, RAC does not consider the neurotoxicity effects relevant for STOT RE classification.

The CLP Regulation states that small changes in clinical biochemistry and haematology are not sufficient to support classification. The CLH dossier contains detailed information about haematological alterations in the 90-day study in rat by inhalation route and variations of ± 10% were reported in the following parameters: prothrombin time, activated partial thromboplastin time, fibrinogen, total protein, albumin, A2-Globulin and A/G ratio. The most drastic reported changes in clinical chemistry were increases of 1.3-1.6-fold in the cholesterol and phospholipid concentration and of 9-13-fold in the Γ-Glutamyl transpeptidase (this last change presumably associated to increase in liver weight and to liver degeneration). Therefore, RAC is of the opinion that haematological and clinical chemistry effects are not relevant for STOT RE classification.

Hyaline droplets in kidney tubules were found in males in the 90-day toxicity study in rats by inhalation route (see a summary of these effects in the table below). However, some slight effects were also reported in control animals and only 4 animals were scored with mild degree and 1 with severe degree at 0.134 mg tetramethrin/L. Higher incidence and severity was reported for animals exposed to 0.824 mg/L but this concentration is 4 times higher than the limit concentration in the CLP criteria for classification in Category 2. The effect was not reported in females or any other repeated toxicity studies. Thus, after analysis of all these evidences, RAC does not consider nephrotoxicity as relevant for STOT RE classification.

Table: Summary table of selected adverse effects in the 3-month inhalative study in rats (Kawaguchi, 1991).

		Dose groups			
	SEX	**0 mg/L**	**0.02 mg/L**	**0.134 mg/L**	**0.824 mg/L**
Liver weight (absolute, g)	M	14.20±1.95	15.58±2.53	16.00±1.48*	17.68±2.21**
	F	7.48±0.89	7.96±1.34	8.78±0.81**	10.24±0.64**

Liver weight (relative, %)	M	2.71±0.16	2.98±0.22*	3.42±0.19**	4.19±0.37**
	F	2.57±0.14	2.77±0.29*	3.21±0.07**	3.97±0.27**
Gross pathological changes					
Liver: Dark red	M	0	0	4	9
	F	0	0	1	7
Liver: Soft	M	0	0	3	3
	F	0	0	0	1
Liver: Large	M	0	0	4	6
	F	0	0	2	7
Histopathological examination					
Liver: Focal necrosis	M	1	0	0	3
	F	0	0	0	0
Liver: Massive necrosis	M	0	0	0	0
	F	0	0	0	1
Liver: Hepatocelluar hypertrophy	M	0	0	5 (slight)	5 (slight)
	F	0	0	2 (slight)	9 (slight)
Liver: Bile duct hyperplasia	M	0	0	0	4 (slight)
	F	0	0	0	1 (slight)
Kidney: hyaline droplets in tubules	M	2 (slight)	4 (slight)	3 (slight) 4 (mild) 1 (severe)	2 (slight) 3 (mild) 5 (severe)
	F	0	0	0	0

Number of affected animals is shown. In all cases the number of examined animals was 10.
*=Significantly different from vehicle control $p < 0.05$; **=Significantly different from vehicle control $p < 0.01$

The hepatotoxicity was consistently reported in most of the repeated dose toxicity studies (see tables above). However, in all cases the effects appeared above the guidance limit concentration for warranting classification as STOT RE category 2 (CLP, Annex I, table 3.9.2), except in the cases of the 90-day toxicity studies by oral route and the 90-day toxicity studies by inhalation route. The oral study describes the effects at 76 mg/kg bw/d as increases in relative liver weight and hypertrophy of hepatocytes both in males, but without reporting incidence.

The effects on liver reported in the inhalation study are summarised in the table above. It shows that at 0.134 mg tetramethrin/L the maximum increases in the liver weight and relative liver weight was 18% (females) and 26% (males), respectively. Males seem to be more susceptible than females to tetramethrin-induced hepatotoxicity since between 30-40% of the animals displayed gross pathological changes, and in addition hepatocellular hypertrophy were reported in 50% of male animals. Higher incidence and severity in liver impairments were reported for animals exposed to 0.824 mg/L but this concentration is outside the classification guidance concentration.

RAC noted that the severe effects in liver always appeared above the maximum guidance doses/concentrations for warranting classification for STOT RE Category 2. Only in a few cases the hepatotoxicity was reported below these limits and always with low incidence and slight/mild severity. Thus, using a weight of the evidence approach, RAC considered the hepatotoxicity not relevant for classification purposes for STOT RE.

In conclusion, RAC agreed with the DS that d-trans-tetramethrin **does not meet the criteria for classification for STOT RE.**

10.14 ASPIRATION HAZARD

Table 65: Summary table of evidence for aspiration hazard

Type of study/data	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
Not applicable				

RAC evaluation of aspiration toxicity

Summary of the Dossier Submitter's proposal

The DS did not include information for this hazard in the CLH report.

Comments received during public consultation

No comments were received during public consultation on this hazard class.

Assessment and comparison with the classification criteria

There is no available information that would enable the potential for aspiration toxicity of tetramethrin to be assessed. In addition, this hazard category is relevant only for certain liquids of low viscosity, while tetramethrin is described as a solid.

11. EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 ACUTE AQUATIC HAZARD

The applicant provided ecotoxicological tests performed with tetramethrin for the effect assessment of d-trans-tetramethrin. Both substances consist of the same 4 isomers (1R trans, 1R cis, 1S trans, 1S cis). However, there is a difference in the ratio of the isomers. Tetramethrin contains the isomers 1R trans, 1R cis, 1S trans, 1S cis in the ratio 4:1:1:4. D-trans-tetramethrin contains mainly the isomer 1R trans with > 90 %. In general, different isomeric mixtures may result in different ecotoxicological effect values dependant on which isomer is the most active and on the composition of the mixture. Compilation of the available data gives the following picture:

Table 66:

	Fish (LC_{50})	**Daphnia (EC_{50})**	**Algae (E_rC_{50})**
tetramethrin	3.7 µg/L	0.11 mg/l	> 0.25 mg/l
d-trans-tetramethrin	5.9 µg/L	-	> 1.25 mg/L

For fish (*O. mykiss*) and green algae effect values for both compounds are available. The LC_{50}/EC_{50} values are quite similar. Although the data basis is quite scarce, it is concluded that both compounds are ecotoxicologically equivalent and the effect values for tetramethrin are used for the effects assessment of d-trans-tetramethrin. The table below summarises the available key studies.

Table 67: Summary of relevant information on acute aquatic toxicity

Method	Species	Test (endpoint, design, duration)	Results[1]	Key or Supportive study	Remarks	Reference
OECD 203; FIFRA 72-1; OPPTS 850.1075	*Oncorhyncus mykiss*	mortality flow-through 96 h	**LC_{50} = 5.9 µg/l** (m)	key	Solvent DMF used	York D., 2008 d-trans-Tetramethrin_14.4.1_03_short-term toxicity fish
EPA OPP 72-2	*Daphnia magna*	Immobility flow-through 48 h	**EC_{50} = 0.11 mg/l** (m)	key	Solvent DMF used; abnomal behaviour observed: surfacing, daphnids on bottom of the vessel, trailing extraneous material, moveing slower; test substance **Tetramethrin.**	Blasberg, 1993 Tetramethrin_14.4.2_02_short-term toxicity invertebrates_Sumitomo
OECD 201	*Pseudokirchneriella subcapitata*	growth inhibition static 72 h	**ErC_{50} > 1.25 mg/l** (MMC) **NOErC = 0.25 mg/l** (m)	key	Solvent DMF used	Hoberg 2002; d-trans-Tetramethrin_14.4.3_growth inhibition algae

[1] Indicate if the results are based on the measured (m) or on the nominal (n) concentration

11.1.1 Acute (short-term) toxicity to fish

An acute fish test using *Oncorhynchus mykiss* as test species was performed with d-trans-tetramethrin as test substance (York, 2008). 10 fish per replicate (2 replicates per concentration) were exposed in a flow-through system to 5 concentrations of the test substance (2.0, 4.0, 8.0, 16 and 32 µg/L (nominal), a control and a solvent control. Dimethylformamide (DMF) was used as a solvent. The solvent control chamber received an aliquot of 0.05 m/L of DMF which was equivalent to that received by the highest test concentration. Mean measured concentrations ranged between 1.6 and 31 µg/L. All effect values are based on mean measured concentrations. Mortality first occurred at a concentration of 7.8 µg/L (mean measured). The 96h-LC_{50} was determined to be 5.9 µg/L. The applicant provided two further acute fish tests with d-trans tetramethrin (cf. study summaries "d-trans-Tetramethrin_14.4.1_01_short-term toxicity fish" and "d-trans-Tetramethrin_14.4.1_02_short-term toxicity fish", see confidential annex). However, both studies by Sousa & LeBlanc (1981) were regarded as not valid by the eCA as both tests were performed in a static test system without analytical monitoring of the test substance concentration. For the ecotoxicological equivalent compound

tetramethrin, a 96h-LC_{50} of 3.7 µg/L was obtained for *Oncorhynchus mykiss* (cf. study summary "Tetramethrin_14.4.1_03_short-term toxicity fish_Sumitomo", see confidential annex).

The lowest available aquatic endpoint for tetramethrin is the LC_{50} = 5.9 µg/l for *Oncorhynchus mykiss*. However, the classification of d-trans-tetramethrin is based on the 96h-LC_{50} of 3.7 µg/L for tetramethrin, as the ecotoxicological equivalence of both compounds was accepted.

Acute (short-term) toxicity to aquatic invertebrates

No acute aquatic toxicity study with invertebrates has been provided by the applicant for d-trans-tetramethrin. Instead, an acute toxicity study with *Daphnia magna* according to EPA guideline was performed for tetramethrin, using a flow-through system (Blasberg, 1993). The test substance concentration was measured at test start and end. 20 daphnids divided in 2 replicates were exposed to 5 test substance concentrations (0.06 – 1 mg/l nominal), a control and a solvent control. DMF was used as solvent. The solvent control chamber received an aliquot of 0.1 m/l of DMF which was approximately equivalent to that received by the highest test concentration. The result is based on mean measured concentration as the measured concentrations significantly deviate from the nominal concentrations The mean measured concentration was 31 ($\pm$5) % of the nominal concentration. The 24h-EC_{50} was > 0.38 mg/l, the highest concentration tested. Although the study was performed with tetramethrin instead of d-trans-tetramethrin, it can be used for the effect assessment of d-trans-tetramethrin, as the available studies with fish and algae allow the conclusion, that the toxicity of both compounds is comparable.

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

An algae growth inhibition test according to OECD 201 is available for d-trans-tetramethrin (Hoberg, 2002). Green algae *Pseudokirchneriella subcapitata* were exposed to 5 test concentrations (0.38, 0.75, 1.5, 3.0 and 6 mg/l nominal), a control and a solvent control (3 replicates each) over 72 h. DMF was used as a solvent. The solvent control chamber received an aliquot of 0.1 ml/l of DMF which was approximately equivalent to that present in the test solutions. Test substance concentration was measured at test start and end. At test start measured concentrations were in the range of 40 – 84 % of nominal. After 72 hours for all test concentrations the concentration was below the limit of detection. Due to the strong deviation between measured and nominal concentrations, the effect values have to be based on mean measured concentration. According to the OECD Guidance Document on Difficult Substances the detection limit should be used, if the concentration at test end is not detectable. Mean values of cell concentration ($x10^4$ cells/ml) were measured at test start and after 24, 48 and 72 hours.The NOE_RC is 0.25 mg/l and the E_RC_{50} > 1.25 mg/l.

11.2 LONG-TERM AQUATIC HAZARD

No long term aquatic data are available for both d-trans-tetramethrin and tetramethrin.

11.3 BIOACCUMULATION

Table 41: Summary of relevant information on bioaccumulation

Method	Species	Results	Key or Supportive study	Remarks	Reference
OECD 305	Bluegill Sunfish (*Lepomis macrochirus*)	827 L*$kg_{wet\ fish}^{-1}$	Key	Information is available for (1RS)-trans-tetramethrin as isomeric mixture, not for the single isomers. But, read across between the both trans-isomers is possible due to information on the optical trans-isomer ratios in the water and fish samples.	Saito S, Miyamoto M, Tagawa Y and Hagino S (1994); Report No. IM-40-0019
QSAR (according to Guidance on the Biocidal Products Regulation, Volume IV)	-	902 L*$kg_{wet\ fish}^{-1}$	Supportive	Log K_{ow}: 4.3 (25°C)	Roth, H. (2006)

11.3.1 Estimated bioaccumulation

Table 68: Estimations on aquatic bioconcentration

Basis for estimation	log P_{OW} (measured)	Estimated BCF for fish (freshwater)	Reference
Standard equation (74), TGD on Risk Assessment (2003), Part II, chapter 3.8.3.2	4.3	902 L*$kg_{wet\ fish}^{-1}$	Roth, H. (2006)

Based on the physicochemical properties an approximate estimation of the bioconcentration factor BCF can be calculated according to Guidance on the Biocidal Products Regulation (2015, volume IV, chapter 3.8.3.2, equation 74, p. 144). Taking into account the log K_{OW} value of 4.3 given by the company Sumitomo the BCF_{fish} amounts to:

$$\text{Log BCF}_{fish} = 0.85 * \log K_{OW} - 0.70$$
$$\text{Log BCF}_{fish} = 0.85 \cdot (4.3) - 0.70$$
$$\text{Log BCF}_{fish} = 2.955$$
$$BCF_{fish} = 902\ L*kg_{wet\ fish}^{-1}$$

K_{OW} Octanol-water partition coefficient
BCF_{fish} Bioconcentration factor for fish on wet weight basis

Summarised the calculated BCF_{fish} for D-trans-tetramethrin amounts to 902 $L*kg_{wet\ fish}^{-1}$, indicating the active substance as potentially bioaccumulative.

11.3.2 Measured partition coefficient and bioaccumulation test data

Table 69: Measurements of aquatic bioconcentration/depuration

Guideline/ Test method	Exposure [days]	Initial concentr. (nominal)	Measured Steady state BCF_L[1]	Calculated Kinetics BCF_L[1]	$T_{1/2}$ for clearance [days]	Identified Metabolites	Reference
US EPA Subdivision N, § 165-4, Laboratory Studies of Pesticide Accumulation in Fish (1982	28	1 µg/L	[Alc-^{14}C]-(1RS)-trans-tetramethrin[2]				SUMI-TOMO: Saito, S., Miyamoto, M., Tagawa, Y. and Hagino, S. (1994); DocNo. IM-40-0019; Doc IIIA 7.4.3.3.1
			827	642	6.06	Whole fish and edible portion: THPI, HPI Non-edible portion: 1 Unknown	
			[Acid-^{14}C]-(1RS)-trans-tetramethrin[2]:				
			722	695	1.37	Whole fish: CRA, COOH-CRA conj.-1 & conj.-2 Edible portion: CRA, CH_2OH-CRA Non-edible portion: CRA, CRA conj., COOH-CRA, COOH-CRA conj.-1 & conj.-2	

[1] BCF values related to total radioactive residues, whole fish and a lipid content of 5%.

[2] 1R-trans-tetramethrin: This isomer is contained with 89 % in the active substance D-trans-tetramethrin.

In consequence of the log K_{OW} above 3 an experimental study with fish is required. A study on bioaccumulation in aquatic organisms is missing for the active substance d-trans-tetramethrin (isomeric ratio: 1R-trans-tetramethrin 89%, 1S-trans-tetramethrin 4%, 1R-cis-tetramethrin 2%, 1S-cis-tetramethrin 0.1%). Instead an aquatic bioaccumulation study with (1RS)-trans-tetramethrin (1:1 mixture of the both trans-isomers) was delivered by the company Sumitomo. An individual BCF_{fish} value for 1R-trans-tetramethrin is lacking. However, taking into account information on the optical trans-isomer ratios in the samples of the available study the BCF value derived for (1RS)-trans-tetramethrin can be used for the environmental risk and hazard assessment of d-trans-tetramethrin (explanation see below).

A dynamic 42-day study was conducted to evaluate the accumulation and the elimination of (1RS)-trans-tetramethrin as well as its metabolism in bluegill sunfish (*Lepomis macrochirus*). All test fish were from the same year class (less than 1 year of age) and held 8 months prior to testing. Their initial

mean body weight was 1.9±0.2 g, they had an initial mean standard body length of 4.1±0.1 cm, and a mean fat content of 3.7%. The fish sampled during the study had a body weight and a standard body length of 2.49±0.47 g and 4.4±0.2 cm. Further information on weight, length and fat content are not available. Due to this a growth correction of the resulting BCF values is not possible (see Annex V of OECD 305). The lipid normalisation to 5% was related to the initial mean fat content of 3.7% as further information on fat content is not available. In the uptake phase 85 fish were exposed each to either [Alc-^{14}C]- or [Acid-^{14}C]- (1RS)-trans-tetramethrin at the concentration of 1 µg/L under a flow-through test condition for 28 days at 25 °C. The measured concentrations for ^{14}C and (1RS)-trans-tetramethrin in water were 0.899 – 1.10 µg/L and 0.549 – 0.766 µg/L for [Alc-^{14}C] preparation, and 0.922 – 1.10 µg/L and 0.536 – 0.792 µg/L for [Acid-^{14}C] preparation, respectively. On day 28 the fish were transferred to fresh running water to determine elimination rates. For analysis of the ^{14}C compounds in fish, 3 fish for whole fish analysis and 3 additional fish for edible portion (body, muscle, skin and skeleton) and non-edible portion (fin, head and internal organs) analyses were randomly picked up from the treated aquaria on days 3, 7, 14, 21 and 28 of exposure. On days 1, 3, 7, 10 and 14 of the recovery, three fish for whole fish analysis and additional 3 fish for edible portion and non-edible portion analyses were randomly sampled from the treated and control aquaria.

For whole fish and total radioactivity the uptake rate and depuration rate constants were estimated with BIOFAC at 54.2 L kg^{-1} day^{-1} in fish and 0.114 day^{-1} for [Alc-^{14}C]- (1RS)-trans-tetramethrin and 260 L kg^{-1} day^{-1} and 0.506 day^{-1} for [Acid-^{14}C]- (1RS)-trans-tetramethrin, respectively.

The values of BCF_{SSL} and the kinetic BCF_L related to total radioactive residues, whole fish and a lipid content of 5% for [Alc-^{14}C]- (1RS)-trans-tetramethrin amounts to 827 and 642 L*kg$_{wet\ fish}^{-1}$, respectively.

The values of BCF_{SSL} and the kinetic BCF_L related to total radioactive residues, whole fish and a lipid content of 5% derived for [Acid-^{14}C]- (1RS)-trans-tetramethrin amounts to 722 and 695 L*kg$_{wet\ fish}^{-1}$, respectively.

A stable steady state concentration was reached in the study conducted with [Alc-^{14}C]-(1RS)-trans-tetramethrin, but not in the study conducted with [Acid-^{14}C]- (1RS)-trans-tetramethrin. As additionally the BCF values derived from the study conducted with [Alc-^{14}C]- (1RS)-trans-tetramethrin are higher than the BCF values derived for [Acid-^{14}C]- (1RS)-trans-tetramethrin the environmental risk and hazard assessment for d-trans-tetramethrin will be based on the BCF values determined for [Alc-^{14}C]- (1RS)-trans-tetramethrin as a worst-case approach.

The BCF_{SSL} results with 827 L*kg$_{wet\ fish}^{-1}$ in a higher value than the BCF_{KL} with 642 L*kg$_{wet\ fish}^{-1}$. Due to this the BCF_{SSL} of 827 L*kg$_{wet\ fish}^{-1}$ related to total radioactive residues, whole fish and lipid content of 5% derived for (1RS)-trans-tetramethrin will be used as a worst case in the environmental risk and hazard assessment.

For edible portion and total radioactivity the uptake and depuration rate constants were estimated at 34.0 L kg^{-1} and 0.09 day^{-1} for [Alc-^{14}C] preparation as well as 46.5 L kg^{-1} and 0.270 day^{-1} for [Acid-^{14}C] preparation, respectively. For BCF in edible portion a steady state was only reached in the approach with [Alc-^{14}C] preparation. Moreover, the derived BCF values for the [Alc-^{14}C] preparation are higher than that derived for the [Acid-^{14}C] preparation, so the results from the [Alc-^{14}C] preparation are used for BCF derivation related to edible portion. The BCF_{KL} is with 511 L*kg$_{wet\ fish}^{-1}$ higher than the BCF_{SSL} with 453 L*kg$_{wet\ fish}^{-1}$ and can be used as the worst-case value for edible portion. Both BCF values are normalised to a fat content of 5%.

For non-edible portion and total radioactivity the uptake and depuration rate constants were estimated at 99.1 L kg^{-1} and 0.144 day^{-1} for [Alc-^{14}C] preparation as well as 424 L kg^{-1} and 0.380 day^{-1} for [Acid-^{14}C] preparation, respectively.Regarding BCF in non-edible portion steady state was only reached in the approach with [Acid-^{14}C] preparation. As additionally the resulting BCF values for the [Acid-^{14}C] preparation are higher than that derived for the [Alc-^{14}C] preparation, the BCF derivation for non-edible portion is based on the results derived for [Acid-^{14}C] preparation. The BCF_{KL} is with 1508 L$kg_{wet\ fish}^{-1}$ higher than the BCF_{SSL} with 1338 L*$kg_{wet\ fish}^{-1}$ and represents the worst-case value for non-edible portion. Both BCF values are normalised to a fat content of 5%.*

Despite the study on bioaccumulation was conducted with (1RS)-trans-tetramethrin (1:1 mixture of the both trans-isomers) the derived BCF values can be used for the active substance d-trans-tetramethrin containing mainly the 1R-trans-isomer (isomeric ratio: 1R-trans-tetramethrin 89%, 1S-trans-tetramethrin 4%, 1R-cis-tetramethrin 2%, 1S-cis-tetramethrin 0.1%), if taking the available information on optical trans-isomer ratios in the samples into account for read-across between the both trans-isomers. Measurement of the ratios of the both isomers R-trans-tetramethrin and S-trans-tetramethrin verifies that they remain at ca. 1/1 ratio in water and fish during the study (see table 4-14). This means that the BCF value of 827 L*$kg_{wet\ fish}^{-1}$ derived for (1RS)-trans-tetramethrin is also valid for the single trans-isomers.

Table 70: Optical isomer ratio in the samples

Label position	Sample	R/S ratio
[Acid-^{14}C]	Water (exposure 0 days)	53 / 47
	Water (exposure 28 days)	48 / 52
	Fish, non edible (exposure 21 days)	54 / 46
	Fish, edible (exposure 21 days)	51 / 49
	Fish, whole (exposure 14 days)	40 / 60
	Fish, whole (exposure 28 days)	46 / 54
[Alc-^{14}C]	Fish, non-edible (exposure 21 days)	53 / 47
	Fish, whole (exposure 28 days)	52 / 48

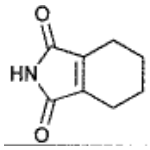
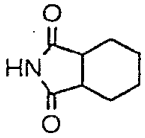
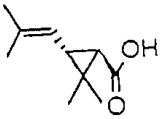
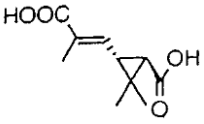
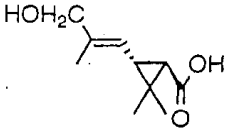
For the reasons described above, the BCF values derived for (1RS)-trans-tetramethrin (1:1 mixture of the both trans-isomers) are also valid for the active substance d-trans-tetramethrin.

The half-life for clearance related to total radioactive residues and whole fish amounts to 6.06 days and 1.37 days for [Alc-^{14}C]- (1RS)-trans-tetramethrin and [Acid-^{14}C]- (1RS)-trans-tetramethrin, respectively. The time to reach 90% of steady-state related to total radioactivity was determined to be 20.1 days for [Alc-^{14}C]- (1RS)-trans-tetramethrin and 4.55 days for [Acid-^{14}C]- (1RS)-trans-tetramethrin.

For edible portion half-life for clearance related to total radioactive residues was determined to be 7.70 days and 2.57 days for [Alc-^{14}C] and [Acid-^{14}C] preparation, respectively. The time to reach 90% of steady-state related to total radioactivity was determined to be 25.6 days for [Alc-^{14}C]- (1RS)-trans-tetramethrin and 8.52 days for [Acid-^{14}C]- (1RS)-trans-tetramethrin.

For non-edible portion half-life for clearance related to total radioactive residues was determined to be 4.81 days and 1.82 days for [Alc-^{14}C] and [Acid-^{14}C] preparation, respectively. The time to reach 90% of steady-state related to total radioactivity was determined to be 16.0 days for [Alc-^{14}C]- (1RS)-trans-tetramethrin and 6.06 days for [Acid-^{14}C]- (1RS)-trans-tetramethrin.

Table 71: List of metabolites found in bioaccumulation study with (1RS)-trans-tetramethrin in whole fish, edible and non-edible portion:

Name of compound used in reports	Structural formula	Maximum concentration[1]	Concentration at end of uptake phase (day 28)[1]	Concentration at end of depuration phase (day 7 or day 10)[1]	Related to:
THPI (3,4,5,6-tetrahydrophthalimide)		13.1 % (day 3)	2.3%	0.3%	**Whole fish**
		22.0% (day 3)	3.3%	0.2%	**Edible portion**
		-	-	-	**Non-edible portion**
HPI (cyclohexane-1,2-dicarboxyimide)		13.0 % (day 3)	2.4%	n.d.	**Whole fish**
		20.8% (day 3)	3.6%	n.d.	**Edible portion**
		-	-	-	**Non-edible portion**
CRA (1R, 3R)-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane-carboxylic acid)		20.1% (day 3)	8.0%	n.d.	**Whole fish**
		50.4% (day 3)	11.0%	n.d.	**Edible portion**
		16.1% (day 3)	5.9%	n.d.	**Non-edible portion**
CRA conj.		-	-	-	**Whole fish**
		-	-	-	**Edible portion**
		11.2% (day 7)	4.5%	n.d.	**Non-edible portion**
COOH-CRA (1R, 3R)-2,2-dimethyl-3-(E-2-carboxyprop-1-enyl)cyclopropane-carboxylic acid)		-	-	-	**Whole fish**
		-	-	-	**Edible portion**
		10.6% (day 7)	5.3%	3.4%	**Non-edible portion**
COOH-CRA conj.-1		15.6% (day 7)	5.2%	n.d.	**Whole fish**
		-	-	-	**Edible portion**
		21.1% (day 7)	8.1%	n.d.	**Non-edible portion**
COOH-CRA conj-2		10.8% (day 7)	4.9%	n.d.	**Whole fish**
		-	-	-	**Edible portion**
		11.3% (day 3)	7.8%	n.d.	**Non-edible portion**
CH_2OH-CRA (1R, 3R)-2,2-dimethyl-3-(E-2-hydroxymethylprop-1-enyl)cyclo-propanecarboxylic acid)		-	-	-	**Whole fish**
		14.6% (day 7, inclusive unknown metabolite A)	9.4%	n.d.	**Edible portion**
		-	-	-	**Non-edible portion**
Unknown (14 unidentified spots pooled)	-	-	-	-	**Whole fish**
		-	-	-	**Edible portion**

Name of compound used in reports	Structural formula	Maximum concentration[1]	Concentration at end of uptake phase (day 28)[1]	Concentration at end of depuration phase (day 7 or day 10)[1]	Related to:
		10.1% (day 3 of depuration phase, max. of single 14 unidentified spots)	Not applicable	3.3%	**Non-edible portion**

The metabolites in whole fish, edible and non-edible portions were analysed by TLC for the fish samples on days 3, 7, 14, 21, and 28 of exposure (see table 4-15). Metabolites detected for both ^{14}C preparations in whole fish throughout the uptake phase with ≥10 % of total radioactivity are THPI (max. 13.1%, day 3), HPI (max. 13.0%, day 3), CRA (max. 20.1%, day 3) and COOH-CRA conj.-1 (max. 15.6%, day 7) and conj.-2 (max. 10.8%, day 7). All metabolites detected with ≥10 % in whole fish decreased to levels between 2.3 and 8.0 at the end of uptake phase and 0.3% and not detectable between day 7 and day 10 of depuration phase. In edible portion 4 metabolites ≥10% were detected for both ^{14}C preparations during the uptake phase, THPI (max. 22.0%, day3), HPI (max. 20.8%, day 3), CRA (max. 50.4%, day 3) and CH_2OH-CRA with unknown metabolite A (max. 14.6%, day 7). At the end of the uptake phase all metabolites were observed with 3.3 – 11.0%. After 7 – 10 days of depuration levels of these metabolites decreased to 5.3% and not detectable in edible portion. In non-edible portion detected metabolites ≥10 % during the uptake phase are Unknown (maximum value of the single 14 unidentified spots pooled in Unknown is 10.1%, day 3 of depuration phase), CRA (max. 16.1%, day 3), CRA conj. (max. 11.2%, day 7), COOH-CRA (10.6%, day 7), COOH-CRA conj.-1 (21.1%, day 7) and COOH-CRA conj.-2 (11.3%, day 3) for both ^{14}C preparations. All these metabolites decrease to levels between 4.5 – 8.1% at the end of uptake phase and 3.4% and not detectable after 7 – 10 days of depuration. Based on these results further investigation on the bioaccumulation behavior of the observed metabolites seems not to be necessary.

Information about metabolites detected in the bioaccumulation study conducted with (1RS)-trans-tetrametrin may also be used for the assessment of the active substance d-trans-tetramethrin as it is not expected that the metabolisation process will result in building of different metabolites for the single isomers. However, it cannot be excluded that the built amounts will be different for d-trans-tetramethrin.

Despite the study on bioaccumulation was conducted with (1RS)-trans-tetramethrin (1:1 mixture of the both trans-isomers) the derived BCF values can be used also to assess the bioaccumulation behaviour of the cis-isomers contained in the active substance d-trans-tetramethrin, if taking the available information on toxicokinetics of the cis- and trans-isomers in rat into account for read-across between cis- and trans-isomers. Toxicokinetics of the cis- and trans-isomers of tetramethrin in rat were almost identical. For details see chapter 3.2 “Toxicokinetics, Metabolism and Distribution”. Minor differences relating to the relative amount of radiocarbon metabolites excreted via urine and the initial elimination velocity were observed for the trans- and cis-isomers. Very rapid systemic and/or pre-systemic metabolic degradation by oxidation and ester hydrolysis has been shown which is slower for cis- than for trans-isomers. However, for cis- as well as trans-isomers a rapid elimination from the body could be observed with excretion of >94% of the dosed ^{14}C into faeces and urine within 2-7 days. As only observed difference trans-isomers are more excreted via urine (trans: 42 – 74%, cis: 9 – 49%) and cis-isomers more via faeces (trans: 21 – 58%, cis: 45 – 91%). For cis- and trans-

isomers the remaining ^{14}C tissue residues were widely distributed with highest concentrations in blood cells and generally low, with 0.2 – 0.4% after 7 days.

The major metabolic pathway of [Alc-^{14}C]-trans-tetramethrin was confirmed to be common between fish and rat. Following cleavage of the ester linkage, the liberated alcohol moiety was further transformed by N-dealkylation, followed by reduction of the 1,2-double bond and/or cleavage of the imide linkage. Taking the information on similarity of rapid elimination from the body and low remaining ^{14}C tissue residues of cis- and trans-isomers in rat as well as the common major metabolic pathway shown for the trans-isomer in rat and fish into account, read-across between cis- and trans-isomers related to bioaccumulation behaviour in fish seems reasonable. Additionally, the similar K_{OC} values of the cis- (2045 mL/g) and trans-isomers (2754 mL/g) may indicate, that BCF values of the trans- and cis-isomers might also be similar. QSAR calculation related to single isomers for support of this conclusion is not possible, as SMILES codes are unable to indicate isomerism. More complex structure activity investigations were not possible as well, due to non availability of suitable tools.

In a publication of Corcellas et al. (Environment International 75 (2015) 110–116) bioaccumulation of 12 pyrethroides including tetramethrin were investigated in wild river fish collected in 4 different Iberian rivers. Pyrethroids were detected in all analysed samples; detection frequency of tetramethrin was 83%. Evaluation of the enantiomeric contribution of tetramethrin was not possible, but in general an accumulation preference of the cis-isomers could be observed according to this publication. Levels of pyrethroids was compared with those of other pollutants like flame retardants, personal care products, hormones and pharmaceuticals. Pyrethroids were detected more frequently in the samples than most other compounds and were also found in higher concentrations. The results of this publication should be kept in mind in future in case of renewal of the authorization procedure. Until then maybe further information might be available on monitoring data regarding tetramethrin and its isomers that should then be taken into account for the environmental risk and hazard assessment.

Summarised measured data on bioaccumulation behaviour in fish are only available for (1RS)-trans-tetramethrin. Taking the available information on optical trans-isomer ratios in the samples into account read-across between the both trans-isomers related to bioaccumulation behaviour in fish seems reasonable. Considering the information on similarity of cis- and trans-isomers regarding K_{OC} values, rapid elimination from the body and low remaining ^{14}C tissue residues in rat as well as the common major metabolic pathway shown for the trans-isomer in rat and fish into account, read-across between cis- and trans-isomers related to bioaccumulation behaviour in fish seems also to be acceptable. Therefore, a BCF_{SS} of 827 $L*kg_{wet\ fish}^{-1}$ related to total radioactive residues, whole fish and lipid content of 5% will be used as a worst case in the environmental risk and hazard assessment for d-trans-tetramethrin. Based on this result d-trans-tetramethrin has to be considered as bioaccumulative.

A test with an appropriate invertebrate species (Doc III-A 7.4.3.3.2) was not required because no direct release to marine/brakish waters occurs. If once the application scenario is changing and release to marine/brackish water occurs an accumulation study with invertebrates might be necessary.

11.4 RAPID DEGRADABILITY OF ORGANIC SUBSTANCES

Table 72: Summary of relevant information on rapid degradability

Method	Results	Key or Supportive study	Remarks	Reference
OECD 301 F	Not readily biodegradable (27% degradation within 28 days)	Key	None	Grūtzner, I. (2002); DocNo SVM-0001

11.4.1 Ready biodegradability

Table 73: Ready biodegradability tests

Method /Guide-line	Test type[1]	Test para-meter	Inoculum			Additional substrate	Test substance conc.	Degradation		Reference
			Type	Conc.[3]	Adap-tation			Incuba-tion period	Degree [%]	
OECD 301 F	ready	BOD[2]	Acti-vated sludge - pre-domi-nantly do-mestic	30 mg/L dw	No	No	104 & 100 mg/L	28 days	27	SUMITOMO: Grützner, I (2002); DocNo SVM-0001; Doc IIIA 7.1.1.2.1

[1] Test on inherent or ready biodegradability according to OECD criteria
[2] Biochemical Oxygen Demand
[3] Suspended solid concentration

A test on ready biodegradability of d-trans-tetramethrin was submitted from the company Sumitomo conducted according to OECD testguideline 301 F investigating ready biodegradability by measurement of biochemical oxygen demand. In this test a biodegradation of 27 % within 28 days was observed. The beginning of relevant biodegradation processes could be observed after 15 days. This delayed degradation may be a sign of adaption procedures.

Based on this result d-trans-tetramethrin can be considered to be not readily biodegradable.

11.4.2 BOD_5/COD

BOD_5/COD tests are not available.

11.4.3 Other convincing scientific evidence

Aquatic simulation tests are not available.

11.4.3.1 Field investigations and monitoring data (if relevant for C&L)

Field investigations and monitoring data are not available.

11.4.3.2 Inherent and Enhanced Ready Biodegradability tests

Inherent and enhanced biodegradability test data are not available.

11.4.3.3 Soil and sediment degradation data

In a laboratory study (OECD 307) the route and rate of aerobic degradation of [^{14}C]-tetramethrin in three soils was investigated using a radiolabel at the alcohol moiety of the molecule. As also the degradation of the single isomers (1R-trans-tetramethrin: contained with 89 % in d-trans-tetramethrin) was determined the results of this study can be used for the assessment of the active substance d-trans-tetramethrin. The first-order DT_{50} values (12°C) were calculated to be 4.0 – 8.0 days for the isomeric mixture tetramethrin, 4.4 – 7.6 days for the isomer 1R-trans-tetramethrin (contained with 89 % in the active substance d-trans-tetramethrin), 2.5 – 4.9 days for 1S-trans-tetramethrin, 15.2 – 36.4 days for 1R-cis-tetramethrin and 13.3 – 21.6 days for the isomer 1S-cis-tetramethrin. Mineralisation of the active substance tetramethrin accounted for a maximum of 57.6 % AR (applied radioactivity) for the silt loam soil (day 30), 33.3 % AR for the loam soil (day 62) and 40.7 % AR for the sandy clay loam soil (day 30). These mineralisation values can also be used for assessment of the active substance d-trans-tetramethrin as the degradation kinetics are very similar between the isomer 1R-trans-tetramethrin (contained with 89 % in d-trans-tetramethrin) and the isomeric mixture tetramethrin containing additionally the slower degradable cis-isomers. Therefore, a similar carbon dioxide evolution is expectable.

In a second laboratory study (US-EPA 162-1) the route and rate of aerobic degradation of [^{14}C]-(1RS)-trans-tetramethrin (1:1 mixture of the both trans-isomers) labeled at the alcohol as well as on the acid moiety of the molecule was investigated. As the DT_{50} values determined for both trans-isomers of tetramethrin in the first soil simulation study are very close together (4.4 – 7.6 days and 2.5 – 4.9 days) the results of the study conducted with (1RS)-trans-tetramethrin can be used for the assessment of the active substance d-trans-tetramethrin.

In the sandy loam soil (1RS)-trans-tetramethrin undergoes degradation under aerobic dark conditions with first order DT_{50} values of 35.6 – 50.6 days (12°C). Carbon dioxide evolution amounts to 49.6% and 49.8% after 122 days of incubation and to 69.8% and 65.0% after 365 days of incubation for [THP-^{14}C]- (1RS)-trans-tetramethrin and [Cyclopropyl-^{14}C] – (1RS)-trans –tetramethrin, respectively. A similar mineralisation rate is expectable for the active substance d-trans-tetramethrin compared to (1RS)-trans-tetramethrin due to the similar degradation kinetics.

Although the results of the simulation studies demonstrate a rapid to moderate primary degradation, the ultimate degradation has to be considered as rather low due to mineralisation rates below 60% (33.3 – 57.6%) after 30 to 122 days of incubation and below 70% (65.0 – 69.8%) after 365 days of incubation. This supports the classification met by the screening test that d-trans-tetramethrin must be considered as not readily biodegradable.

11.4.3.4 Hydrolysis

Table 74:

Method /Guideline	pH	Temperature [°C]	Initial con-centration, C_0, [µg/L]	Reaction rate constant, K_h [$days^{-1}$]	Half-life, DT_{50}	Coefficient of correlation, r^2	Reference	Key or Supportive study
Trans-Neo-Pynamin [3,4,5,6-tetrahydrophthalimidomethyl (1RS)-trans-chrysanthemate or (1RS)-trans-tetramethrin] US EPA Pesticide Assessment Guidelines Subdivision N 161-1	5	25	300	$3.514x10^{-2}$ – $4.364x10^{-2}$	15.9-19.7 days	n.s.	Katagi T. et al, Sumitomo Report No: IM-10-0012, 1991	Key
	7	25	300	0.653 – 0.778	21.4-25.5 hrs	n.s.		
	9	25	300	44.2 – 75.6	13.2-21 min	n.s.		

A definitive hydrolysis study on degradation products and kinetics was conducted with (1RS)-trans-tetramethrin at pH 5, 7 and 9 at 25 °C according to US EPA N 161-1. The temperature dependence of hydrolysis has not been determined in this study. Information is only available for (1RS)-trans-tetramethrin, information on the single (1R)-trans-isomer is lacking. The eCA does not expect enantiomerism to affect significantly the hydrolysis of the parent compound under environmental conditions and, hence, considers the data as sufficient to characterise the route and kinetic of degradation and the mayor degradation products of the (1R)-trans-isomer.

The mayor degradation products of (1RS)-trans-tetramethrin are (1RS)-trans-crysamtemic acid (trans-CRA) and 3,4,5,6-tetrahydrophthalamic acid (THAM), that is finally hydrolysed to 3,4,5,6-tetrahydrophthalic acid (THPA). Trans-CRA increased to 68.85% of the applied radioactivity at pH 5, 98.42% at pH 7 and 100% at pH 9 at day 30. THMA peaked after one day to 15.43% at pH7 and to 80.6% at pH9. THPA increased to 66.07% at pH5, 95.78% at pH 7, and 68.07 % at pH9. The degradation products trans-CRA and THPA are assumed to be hydrolytic stable.

The hydrolysis half-lives of (1RS)-trans-tetramethrin were recalculated to reflect an average EU outdoor temperature of 12°C for fresh water (based on EU TGD (2003), chapter 2.3.6.1). The half-lives amount to 45.0 – 55.7 days at pH 5, 66.5 – 72.1 hours at pH 7, and 37.3 – 59.4 min at pH 9.

11.4.3.5 Photochemical degradation

Photolysis in water

Table 75:

Method /Guideline	Initial molar TS concentration	Total recovery of test substance [% of appl. a.s.]	Photolysis rate constant (k^c_p)	Direct photolysis sunlight rate constant (k_{pE})	Reaction quantum yield (Φ^c_E)	Half-life DT_{50} [days]	Reference	Key or Supportive study
US EPA OPPTS 835.2210	0.2 µg/mL	95.6±7.0%	0.147 h^{-1}	n.s.	0.19	0.46 US summer days; 0.38 Global summer days	Lopez A., PTRL Report No. 1762W-1, 2003	Key

(1R)-trans-tetramethrin undergoes photodegradation in aqueous media at pH 5. A degradation rate constant of 0.147 $hours^{-1}$ and a half-life of DT_{50} = 0.46 days for a US summer day was determined. Photo-induced isomerisation to the cis-isomer was minor in light exposed samples. The other main degradation products observed were not known and were assigned as D-1, D-3 and D-6. Based on LC/MS data proposed structures are given (see confidential annex *d-trans-Tetramethrin_14.4.17_03*). D-1 represented an average of 24.1% of the initial dose at the end of the irradiation period (304 hours). D-3 and D-6 reached after 144 hours of irradiation 32.2% and 20.1% of the applied dose, respectively.

(1R)-trans-tetramethrin degraded significantly in dark control samples. After 312 hours of incubation at 25°C, 47% of the initial dose occurred in the non-irradiated samples. The major degradation product was THPA, reaching an average of 55.7% of the applied dose at the end of the incubation period. The corresponding half-life in dark controls was 12.3 days of incubation. Since (1R)-trans-tetramethrin degraded more rapidly when exposed to light, its hydrolytic degradation without irradiation had no significant effect on the light exposed set.

Indirect photolysis in water bodies of the active substance has not been measured. However, information on indirect photolysis is not regarded to be scientifically necessary as other degradation process (hydrolysis, direct photolysis) are not regarded to be slow.

Phototransformation in air

Table 76:

Guideline / Test method	Time-dependent OH radical concentration [OH radicals cm^{-3}]	Overall reaction rate constant k [cm^3 $molecule^{-1}$ s^{-1}]	Half-life DT_{50} [h]	Chemical lifetime [h]	Reference	Key or Supportive study
AOPWIN calculation v. 1.92	$5x10^5$ 24 h avarage	127.3092 E^{-12}	3.025	4.36	(CA's estimation)	Key

(1R)-trans-tetramethrin is expected to degrade in the atmosphere by reaction with photochemically-produced hydroxyl radicals. The half-life of (1R)-trans-tetramethrin in air is estimated to be 3.025 h using the generally accepted estimation program AOPWIN, version 1.92. Referring to the vapour pressure of 4.03 x 10^{-6} Pa and a Henry's Law constant of 0.000112 Pa m^{-3} mol^{-1} (1R)-trans-tetramethrin is not expected to volatilize. Therefore, emissions to air are expected to be low. In conclusion, due to the low volatility, the fast degradation by OH radicals in air, the hydrolysis in the presence of water, accumulation and long range transport in air are not to be expected under environmentally relevant conditions.

11.5 ENVIRONMENTAL FATE AND OTHER RELEVANT INFORMATION

11.5.1 Adsorption/desorption

Table 77: Adsorption/desorption in five soils

Method /Guideline	Tested Soils	Adsorbed a.s. [%]	K_a[1]	K_{aOC}[2]	K_d [3]	K_{dOC} [4]	K_a / K_d[5]	Degradation products	Reference	Key or Supportive study
US EPA Pesticide Assessment Guidelines Subdivision N 163-1	A13 = Sandy loam	64.4	11.47	1289	13.61	1529	0.84	none	Yoshimura J., Sumitomo Report No: IM-10-0013, 1991	Key
	B7 = Loamy sand	59.7	12.03	2933	12.99	3167	0.93			
	F6 = Sandy clay loam	59.9	19.27	1407	21.06	1537	0.92			
	Hanford = Sandy Loam	47.2	8.54	1857	8.03	1746	1.06			
	J11= Clay	59.3	10.71	1552	11.92	1728	0.90			

[1] K_a = Adsorption coefficient, mean value of duplicate measure
[2] K_{aOC} = Adsorption coefficient based on organic carbon content, mean value of duplicate measure
[3] K_d = Desorption coefficient, mean value of duplicate measure
[4] K_{dOC} = Desorption coefficient based on organic carbon content, mean value of duplicate measure
[5] K_a / K_d = Adsorption / Desorption distribution coefficient, mean value of duplicate measure

A study on adsorption/ desorption of (1RS)-trans-tetramethrin in five different soils was conducted according to EPA 163-1. Information is only available for (1RS)-trans-tetramethrin, information on the single (1R)-trans-isomer is lacking. The eCA does not expect enantiomerism to affect significantly the sorption behaviour of the parent compound in soils and, hence, considers the data as sufficient to characterise the adsorption/desorption behaviour of the (1R)-trans-isomer. Nevertheless, deviations from the OECD guideline 106 were determined regarding reporting on soil sampling and use of standard methods for soil characterization. Deficiencies were further determined regarding soil classification. Moreover, deficiencies were determined regarding the range of the five different soils

as the selection does not comprises acidic soil types with a pH <6.5, soil types of high OC content >1.5% and the soil types do not comprise five different soils according to the OECD guideline 106.

Radiolabeld (1RS)-trans-tetramethrin was stable in the test system during the study as more than 99 % was identified to be (1RS)-trans-tetramethrin at the end of the study. The adsorption equilibrium coefficient Ka ranged from 8.54 to 19.32 mL/g and the desorption equilibrium coefficient Kd ranged from 8.03 to 21.06 mL/g. The Ka_{oc} value was calculated to range from 1289 to 2933 mL/g and the Kd_{oc} value was calculated to range from 1529 to 3167 mL/g. K_{oc} values did not correlate with the OC content. The arithmetic mean K_{oc} is 1807 mL/g, with a mean log K_{oc} of 3.26 mL/g. From the average K_{oc} values for adsorption and desorption it can be concluded that (1RS)-trans-tetramethrin is considered of low mobility in four types of soil and as slight mobility in one soil type tested.

11.6 COMPARISON WITH THE CLP CRITERIA

11.6.1 Acute aquatic hazard

For d-trans-tetramethrin an acute aquatic endpoint for fish is available, for invertebrates a read-across to Tetramethrin was accepted. For algae a test according to OECD 201 was done and an ErC_{50} and a NOErC were derived. The most sensitive endpoint is a LC_{50} = 5.9 µg/l for *Oncorhynchus mykiss* (cf. chapter 11.1.1). However, the classification of d-trans-tetramethrin is based on the 96h-LC50 of 3.7 µg/L for tetramethrin, as the ecotoxicological equivalence of both compounds was accepted. A substance has to be classified as "Aquatic Acute 1; **H400**", if the LC_{50}/EC_{50} is ≤ 1 mg/l. This criterium is fulfilled for d-trans-tetramethrin. The corresponding **M-factor is 100**.

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

D-trans-tetramethrin has shown a biodegradation of only 27% in 28 days in a test according to OECD guideline 301 F and has therefore to be regarded as not readily biodegradable (cf. chapter 11.4.1). With an estimated BCF_{fish}, based on the log K_{ow} = 4.3, of 902 $L*kg_{wet\ fish}^{-1}$ and a measured BCF_{fish} value of 827 $L*kg_{wet\ fish}^{-1}$ (cf. chapter 11.3) d-trans-tetramethrin is considered to be bioaccumulative. The "Guidance on the Application of the CLP Criteria" (ECHA, 2013) gives a decision tree for the decision on categories for substances long-term hazardous to the aquatic environment (Figure 4.1.1, p. 524). According to this decision tree, if adequate chronic toxicity data are only available for one or two trophic levels, the chronic classification should be done considering both the available chronic endpoints and also, as a surrogate system, considering the acute endpoints. The most stringent outcome should be taken for the classification. In the case of d-trans-tetramethrin, long-term aquatic toxicity data are only available for algae, but algae are not the most sensitive organisms in acute toxicity tests. Taking into account the lowest available acute LC_{50} 3.7 µg/L (*Oncorhynchus mykiss*) for the ecotoxicological equivalent substance tetramethrin (read-across was accepted by the Competent Authority), d-trans-tetramethrin has to be classified as "Aquatic Chronic 1; H410.
As there are no chronic effect data available, the acute M-factor of 100 has to be applied for the chronic category as well.

11.7 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

D-trans-tetramethrin should be classified as "Aquatic Acute 1; H400" – "Very toxic to aquatic organisms" (M = 100) and "Aquatic Chronic 1; H410" – "Very toxic to aquatic organisms with long lasting effects" (M = 100) for the environment. This leads to a proposed labelling of H410 (Very toxic to aquatic life with long lasting effects), which triggers the pictogram GHS09 and the signal word "Warning" on the label. The following precautionary statements are indicated: P273, P391 and P501.

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The DS provided ecotoxicological tests performed with tetramethrin for the effect assessment of d-trans-tetramethrin. Both substances consist of the same 4 isomers. Nevertheless, there is a difference in the ratio of the isomers. In general, different isomeric mixtures may result in different ecotoxicological effect values. However, based on the very similar effect values and that the database is quite scarce, the DS concluded that d-trans-tetramethrin and tetramethrin are ecotoxicologically equivalent and that the aquatic effect values for tetramethrin can be fully used for the effects assessment of d-trans- tetramethrin.

As the ecotoxicological equivalence of both substances was accepted, the DS, based on tetramethrin data, proposed an environmental hazard classification as Aquatic Acute 1 (H400) with an M-factor of 100 based on the lowest acute aquatic toxicity to the fish *Oncorhynchus mykiss* (96-h LC_{50} = 0.0037 mg/L).

As chronic/long-term data are not available for fish and invertabrates, the DS proposed d-trans-tetramethrin to be classified as Aquatic Chronic 1 (H410) with an M-factor of 100, based on acute aquatic toxicity to the fish *Oncorhynchus mykiss* (96-h LC_{50} = 0.0037 mg/L) combined with degradation and bioaccumulation data as the most stringent outcome.

Degradation

The ready biodegradation of d-trans-tetramethrin was investigated by one key study (Grützner, 2002) which was conducted according to OECD TG 301 F investigating ready biodegradability by measurement of biochemical oxygen demand (BOD^2). According to the test results, the d-trans-tetramethrin degraded 27% within 28 days. Therefore, based on the CLP criteria, the DS concluded that d-trans-tetramethrin can be considered as not readily biodegradable.

Soil and sediment degradation data are not available for d-trans-tetramethrin.
A definitive hydrolysis study on degradation products and kinetics was conducted with (1RS)-trans-Tetramethrin at pH 5, 7 and 9 at 25 °C according to US EPA N 161-1. The temperature dependence of hydrolysis has not been determined in this study. Information is only available for (1RS)-trans-tetramethrin; information on the single (1R)-trans-isomer is lacking. It is not

expected enantiomerism to affect significantly the hydrolysis of the parent compound under environmental conditions and, hence, the DS considered the data as sufficient to characterise the route and kinetic of degradation and the major degradation products of the (1R)-trans-isomer.

The major degradation products of (1RS)-trans-Tetramethrin are trans-CRA and THAM, that is finally hydrolysed to THPA. Trans-CRA increased to 68.85% of the applied radioactivity at pH 5, 98.42% at pH 7 and 100% at pH 9 at day 30. THMA peaked after one day to 15.43% at pH7 and to 80.6% at pH9. THPA increased to 66.07% at pH5, 95.78% at pH 7, and 68.07% at pH9. The degradation products trans-CRA and THPA are assumed to be hydrolytic stable.
The hydrolysis half-lives of (1RS)-trans-Tetramethrin were recalculated to reflect an average EU outdoor temperature of 12°C for fresh water (based on EU TGD (2003)). The half-lives amount to 45.0 – 55.7 days at pH 5, 66.5 – 72.1 hours at pH 7, and 37.3 – 59.4 min at pH 9.
(1R)-trans-Tetramethrin undergoes photodegradation in aqueous media at pH 5. A degradation rate constant of 0.147 $hours^{-1}$ and a half-life of DT50 = 0.46 days for a US summer day were determined. Photo-induced isomerisation to the cis-isomer was minor in light exposed samples. The other main degradation products observed were not known and were assigned as D-1, D-3 and D-6.

(1R)-trans-Tetramethrin degraded significantly in dark control samples. After 312 hours of incubation at 25°C, 47% of the initial dose occurred in the non-irradiated samples. The major degradation product was THPA, reaching an average of 55.7% of the applied dose at the end of the incubation period. The corresponding half-life in dark controls was 12.3 days of incubation. Since (1R)-trans-Tetramethrin degraded more rapidly when exposed to light, its hydrolytic degradation without irradiation had no significant effect on the light exposed set.
Indirect photolysis in water bodies of the active substance has not been measured. However, information on indirect photolysis is not regarded to be scientifically necessary as other degradation process (hydrolysis, direct photolysis) are not regarded to be slow.

Bioaccumulation

Information on measured BCF_{fish} (*Lepomis macrochirus*) value of 1670 $L/kg_{wetfish}$ (OECD TG 305) is only available for (1RS)-trans-Tetramethrin; information on the single (1R)-trans-isomer is lacking. However, read-across between both trans-isomers is possible due to information on the optical trans-isomer ratios in the water and fish samples (Saito, 1994). The BCF value of 835 $L/kg_{wet\ fish}$ related to total radioactive residues, whole fish and a lipid content of 5% derived from the results of the bioaccumulation study conducted with [Alc-^{14}C]-trans-Neo-Pynamin (equivalent to (1RS)-trans-Tetramethrin), was considered as a worste case. An individual BCF_{fish} value for (1R)-trans-Tetramethrin is lacking. Therefore, the final BCF_{fish} value of 1670 $L/kg_{wet\ fish}$ for d-trans-tetramethrin has been calculated based on the results of the study conducted with (1RS)-trans-Tetramethrin assuming that only (1R)-trans-Tetramethrin has been enriched.

In a supportive QSAR study according to the standard equation of TGD Risk Assessment, a BCF for fish of 902 $L/kg_{wetfish}$ based on the measured Log Kow 4.3 was estimated.

Therefore, with an estimated BCF_{fish} of 902 $L/kg_{wet\ fish}$ and a measured BCF_{fish} value of 1670 $L/kg_{wet\ fish}$ DS considered d-trans-tetramethrin as bioaccumulative according to CLP criteria.

Aquatic Toxicity

Available acute and chronic ecotoxicological tests results for d-trans-tetramethrin are summarised in the following table and sections.

Test organism / guideline, test method	**Short-term result (endpoint)**	**Long-term result (endpoint)**	**Reference**
Rainbow trout (*Oncorhynchus mykiss*) / OECD TG 2003	96-h LC_{50} = 0.0059 mg/L (measured concentrations)	-	York, 2008 d-trans-tetramethrin
Rainbow trout (*Oncorhynchus mykiss*) / OECD TG 2003	96-h LC_{50} = 0.0037 mg/L (measured concentrations)	-	Bowman, 1990 tetramethrin
Daphnia magna / EPA OPP 72-2	48-h EC_{50} = 0.11 mg/L (measured concentrations)	-	Blasberg, 1993 tetramethrin
Selenastrum capricornutum (*Pseudokirchneriella subcapitata*) / OECD TG 201	72-h E_rC_{50} ≥ 1.25 mg/L (measured concentrations)	72-h NOE_rC = 0,25 mg/L (measured concentrations)	Hoberg, 2002 d-trans-tetramethtin
Selenastrum capricornutum (*Pseudokirchneriella subcapitata*) / OECD TG 201	72-h E_rC_{50} ≥ 0.25 mg/L (measured concentrations)	72-h NOE_rC = 0,25 mg/L (measured concentrations)	Hoberg, 2002 tetramethrin

There is no available aquatic toxicity studies with invertebrates for d-trans-tetramethrin, but only with tetramethrin. The lowest available aquatic effect value for d-trans-tetramethrin is the LC_{50} = 0.0059 mg/L for *Oncorhynchus mykiss*. However, the classification of d-trans-tetramethrin is based on the 96h LC_{50} of 0.0037 mg/L for tetramethrin, as the ecotoxicological equivalence of both compounds was accepted.

As there are no aquatic chronic toxicity studies available for fish and invertebrates, the DS considered to use the lowest aquatic acute data combined with degradation and/or bioaccumulation data for aquatic chronic classification. As d-trans-tetramethrin is considered to be not readily biodegradable and has a potential to bioaccumulate, the DS based aquatic chronic classification on the 96h LC_{50} of 0.0037 mg/L for tetramethrin, as the ecotoxicological equivalence of both compounds was accepted.

Comments received during public consultation

One MSCA submitted comments on the environmental part of the DS's proposal. They agree with the proposed classification of d-trans-tetramethrin as Aquatic Acute 1 (M=100) and Aquatic Chronic 1 (M=100), without further justification.

Assessment and comparison with the classification criteria

Degradation

RAC agrees that the d-trans-tetramethrin is not readily biodegradable based on 27% biodegradation in 28 days in a OECD TG 301 F test and therefore does not meet the criteria for being rapidly degrabable in the environment.

Aquatic Bioaccumulation

The measured log Kow value for d-trans-tetramethrin is 4.3, which is above the CLP Log Kow trigger value of ≥ 4. The measured BCF_{fish} value is 1670 $L/kg_{wet\ fish}$ for (1RS)-trans-Tetramethrin , which is above the CLP BCF trigger value of ≥ 500. An individual BCF_{fish} value for (1R)-trans-Tetramethrin is lacking. The measured BCF_{fish} value of 835 $L/kg_{wet\ fish}$ (related to a lipid content of 5%) derived from a study conducted with (1RS)-trans-Tetramethrin and was considered as a worst case. Therefore, the final BCF_{fish} value of 1670 $L/kg_{wet\ fish}$ for d-trans-tetramethrin has been calculated based on the results of the study conducted with (1RS)-trans-Tetramethrin assuming that only (1R)-trans-Tetramethrin has been enriched. The supportive data is estimated BCF_{fish} value for d-trans-tetramethrin of 902 $L/kg_{wet\ fish}$.

Based on the measured BCF value of 1670 $L/kg_{wet\ fish}$, RAC agrees with the DS's conclusion that the substance has a potential to bioaccumulate and should be considered as bioaccumulative.

Aquatic Toxicity

RAC agrees that d-trans-tetramethrin and tetramethrin are ecotoxicologically equivalent and that the effect values for tetramethrin can be used for the effects assessment of d-trans-tetramethrin. RAC would like to emphasise that this assumption can be reviewed if relevant chronic ecotoxicity tests will be conducted in the future, as one of the isomers appears to be more neurotoxic than the others and is the major component of d-trans-tetramethrin. RAC notes that there are no chronic aquatic toxicity data for fish and aquatic invertebrates. RAC concurs that a potential chronic classification should be made for the trophic level with chronic data and compared with that made using the acute toxicity data for the other trophic levels combined with degradation and/or bioaccumulation data. The final classification shall be made according to the most stringent outcome. RAC noted that, as insects are presumably the target organism group, acute toxicity data for an insect species might possibly affect the M-factor, if relevant ecotoxicity data will become available in the future.

Acute toxicity

RAC agrees with the DS that the lowest acute (short-term) effect value for d-trans-tetramethrin was observed for Rainbow trout (*Oncorhynchus mykiss*) with an acute 96 hours LC_{50} of 0.0059 mg/L (mean measured concentrations). However, the classification of d-trans-tetramethrin is based on the 96h LC_{50} of 0.0037 mg/L for tetramethrin, as the ecotoxicological equivalence of both compounds was accepted. The corresponding M-factor is 100.

Chronic toxicity

The lowest mean measured NOE_rC 0.25 mg/L for d-trans-tetramethrin was observed for the algae *Selenastrum capricornutum*. As no chronic data is available for fish and intervebrates, chronic classification needs to be derived based on the results of both acute and chronic

studies, according to the most stringent outcome (surrogate approach). As such, the most stringent value for classification is the acute 96 hours LC_{50} of 0.0037 mg/L for Rainbow trout (*Oncorhynchus mykiss*) and the corresponding M-factor is 100.

Conclusion on classification

D-trans-tetramethrin is considered as not rapidly degradable and fulfils the criteria as bioaccumulative. In agreement with the DS, RAC is of the opinion that d-trans-tetramethrin should be classified as:

Aquatic Acute 1 (H400) with an **acute M-factor of 100**.

Aquatic Chronic 1 (H410) with a **chronic M-factor of 100.**

12. EVALUATION OF ADDITIONAL HAZARDS

12.1 HAZARDOUS TO THE OZONE LAYER

According to Regulation EC (No) 1272/2008 a substance has to be considered as hazardous to the ozone layer if it is listet in Regulation EC (No) 2037/2000. As this is not the case for d-trans-tetramethrin, no additional labelling is necessary.

13. DETAILED STUDY SUMMARIES

See confidential Annexes

14. REFERENCES

Author(s)	Year	Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Unpublished	Owner
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Grūtzner I	2002	Ready biodegradability of S-1723 in a manometric respirometry test, Environmental Chemistry and Pharmanalytics Division, Sumitomo Report No. SVM-0001, GLP, (Un)	Sumitomo Chemical Co., Ltd
Hohberg	2002	Toxicity to the Freshwater Green Alga (*Pseudokirchneriella subcapitata*), Springborn Smithers Laboratories, Sumitomo Report No. SVW-0002	Sumitomo Chemical Co., Ltd
Katagi	1991	Hydrolysis of trans-Neopynamin in buffered aqueous solution, Environmental health science laboratory, Sumitomo Report No. IM-10-0012, 1991	Sumitomo Chemical Co., Ltd

Lentz NR	2008	Final Neo-Pynamin Forte PAI – Determination of the Denisty of a Test Substance Following OECD Guideline 109 and the Official Journal of the European Communities, L383A, Method A.3, Springborn Smithers Laboratories, Study No. 13048.6572, Sumitomo Report No. SVP-0020, GLP, (Un)	Sumitomo Chemical Co., Ltd
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Lentz NR	2008	Final Neo-Pynamin Forte PAI – Determination of Surface Tension using a Ring Tensiometer Following OECD Guideline 115, Springborn Smithers Laboratories, Study No. 13048.6593, GLP, (Un)	Sumitomo Chemical Co., Ltd
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Poncipe	2006	Tetramethrin. Estimation of indirect photolysis in air, Safepharm laboratories	Sumitomo Chemical Co., Ltd
Roth H	2006	Safepharm Laboratories Ltd, , (Un)	Sumitomo Chemical Co., Ltd
Saito S, Miyamoto M, Tagawa Y and Hagino S	1994	Bioconcentration and metabolism of 14C-trans-Neo-Pynamin in Bluegill Sunfish (*Lepomis macrochirus*), Environmental Health Science Laboratory, Sumitomo Report No. IM-40-0019, GLP, (Un)	Sumitomo Chemical Co., Ltd
Schetter J.E.	1999	Final S-1723 - Vapour Pressure, Analytical Services, Sumitomo Report No. SVP-0006, GLP, (Un)	Sumitomo Chemical Co., Ltd
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Sousa and LeBlanc	1981	Acute toxicity of Neo-Pynamin Forte T.G. to Rainbow Trout (*Salmo gairdneri*). EG & G Bionomics, Aquatic Toxicology Laboratory. Sumitomo Report No. IW-11-0006	Sumitomo Chemical Co., Ltd
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15. ANNEXES

Confidential Annexes