Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

tetramethrin (ISO); (1,3-dioxo-1,3,4,5,6,7-hexahydro-2H-isoindol-2-yl)methyl 2,2-dimethyl-3-(2-methylprop-1-en-1-yl)cyclopropanecarboxylate

EC Number: 231-711-6
CAS Number: 7696-12-0

CLH-O-0000001412-86-125/F

Adopted

16 September 2016

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16 September 2016

CLH-O-0000001412-86-125/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **tetramethrin (ISO); (1,3-dioxo-1,3,4,5,6,7-hexahydro-2H-isoindol-2-yl)methyl 2,2-dimethyl-3-(2-methylprop-1-en-1-yl)cyclopropanecarboxylate**

EC Number: **231-711-6**

CAS Number: **7696-12-0**

The proposal was submitted by **Germany** and received by RAC on **5 October 2015.**

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

PROCESS FOR ADOPTION OF THE OPINION

Germany has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **22 December 2015**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **5 February 2016**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Miguel A. Sogorb**

Co-Rapporteur, appointed by RAC: **Žilvinas Užomeckas**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on 16 september 2016 by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitter's proposal	607-RST-00-Y	tetramethrin (ISO); (1,3-dioxo-1,3,4,5,6,7-hexahydro-2H-isoindol-2-yl)methyl 2,2-dimethyl-3-(2-methylprop-1-en-1-yl)cyclopropanecarboxylate	231-711-6	7696-12-0	Carc. 2 Acute Tox. 4 STOT SE 2 Aquatic Acute 1 Aquatic Chronic 1	H351 H332 H371 (nervous system) (inhalation) H400 H410	GHS08 GHS07 GHS09 Wng	H351 H332 H371 (nervous system) (inhalation) H410		M=100 M=100	
RAC opinion	607-RST-00-Y	tetramethrin (ISO); (1,3-dioxo-1,3,4,5,6,7-hexahydro-2H-isoindol-2-yl)methyl 2,2-dimethyl-3-(2-methylprop-1-en-1-yl)cyclopropanecarboxylate	231-711-6	7696-12-0	Carc. 2 Acute Tox. 4 STOT SE 2 Aquatic Acute 1 Aquatic Chronic 1	H351 H302 H371 (nervous system) (inhalation) H400 H410	GHS08 GHS07 GHS09 Wng	H351 H302 H371 (nervous system) (inhalation) H410		M=100 M=100	
Resulting Annex VI entry if agreed by COM	607-RST-00-Y	tetramethrin (ISO); (1,3-dioxo-1,3,4,5,6,7-hexahydro-2H-isoindol-2-yl)methyl 2,2-dimethyl-3-(2-methylprop-1-en-1-yl)cyclopropanecarboxylate	231-711-6	7696-12-0	Carc. 2 Acute Tox. 4 STOT SE 2 Aquatic Acute 1 Aquatic Chronic 1	H351 H302 H371 (nervous system) (inhalation) H400 H410	GHS08 GHS07 GHS09 Wng	H351 H302 H371 (nervous system) (inhalation) H410		M=100 M=100	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Tetramethrin is a biocidal and 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 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)
Sexual function and fertility	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 tetramethrin and d-trans-tetramethrin;

6. NOAEL/LOAEL intervals for tetramethrin and d-trans-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 months	95	325
Dog	6 months	90	180
d-trans-tetramethrin			
Rat	28 days	290 (M) / 295 (F)	965 (M) / 940 (F)
Rat	6 months	58 (M) / 71 (F)	178 (M) / 214 (F)

M=male; F=female

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

8. Neither tetramethrin nor d-trans-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 d-trans-tetramethrin can be used as additional evidence for the assessment of human health hazards of tetramethrin.**

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 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.

Tetramethrin failed to show flammability properties in two different normalised EU A.10 assays and therefore does not meet the criteria for classification as a flammable solid.

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).

The study for testing self-heating substances does not need to be conducted because tetramethrin is a solid having a melting point ≤ 160 °C.

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.

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 normal circumstances. In addition, according to guidelines the solid organic substances containing oxygen chemically bonded only to carbon or hydrogen (as is the case of 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 tetramethrin regarding physico-chemical hazards.**

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification of 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 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 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 OECD TG 403 study performed with tetramethrin 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 Company/Manufacturer submitted comments to clarify the points that allowed the DS to consider the acute rat inhalation study with tetramethrin as less relevant. They pointed out that

the study should be considered fully valid with an LC_{50} higher than 5.63 mg/L due to the following reasons:

- The chamber was allowed to attain the equilibrium for at least 30 minutes before the start of the test;
- The tetramethrin concentration inside the chamber was monitored in four independent measurements at 45, 105, 165 and 220 minutes after the start of the exposure;
- The mean ± SD of the four measurements was 5.63 ± 0.86 mg tetramethrin/L and therefore the test item concentration was constant throughout the exposure period indicating steady state of concentration;
- The presence of dense aerosol inside the chamber did not allow ascertaining the occurrence of clinical signs.

The DS replied that the results of this study strongly contrasts with the other acute inhalation study performed with d-trans-tetramethrin reporting neurotoxicity from concentrations of 0.13 mg/L and one lethality at 1.18 mg/L and with the subacute inhalation study, also with d-trans-tetramethrin, showing similar acute neurotoxicity signs after daily exposures at concentrations of 0.09 mg/L.

One MSCA considered 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.

Assessment and comparison with the classification criteria

In the three tables below, the acute oral, dermal and inhalation animal toxicity studies with tetramethrin are summarised. No cases of poisoning with tetramethrin in humans have been reported.

Table: Summary of acute oral toxicity studies with tetramethrin.
(in all cases the vehicle was 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	LD50 > 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	LD50 > 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 was 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 Head and nose exposure	Rat Wistar Preliminary study (G1): 2 M + 2 F Main study (G2): 5 M + 5 F	5.63 ± 0.86 mg/L 4 hours Aerosol in cyclohexanone (50% w/v); Mean aerosol particle size: G1: 0.67 ± 0.26 µm G2: 0.68 ± 0.26 µm	LC50 > 5.63 mg/L Slight lacrimation and nasal discharge on day 1 (all rats in G2), normal from day 2 onwards, No toxic signs observed	Venugopala, 2006

Comparison with the criteria

The available information for acute oral toxicity of tetramethrin comprises two rat studies causing no mortalities at doses of 5000 mg/kg bw and one study, also in rat, with the analogue d-trans-tetramethrin causing a single mortality at the same dose. Finally, there is a fourth study in mouse with d-trans-tetramethrin showing neurotoxicity and with an LD_{50} of 1050 mg/kg bw (combined for both sex). It suggests that mice might be more sensitive to tetramethrins that rat and, according to the Guidance on the Application of the CLP Criteria (CLP guidance) the most sensitive species should be used for classification. Thus, the LD_{50} in the most sensitive species is higher than 300 and lower than 2000 mg/kg bw/d and consequently RAC concluded that tetramethrin should 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. The available information shows that doses of up to 5000 mg/kg bw of tetramethrin or d-trans-tetramethrin did not cause mortalities. Thus, RAC agreed with the DS that tetramethrin **does not fulfil 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 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 out of 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. The DS also provided a study showing no mortalities in 10 animals after 4 hours of exposure to 5.63 mg tetramethrin/L, however, they did not consider the study appropriate for classification due to technical deficiencies. Nevertheless, RAC notes that Industry during public consultation supplied information demonstrating that the technical concerns exposed by the DS are not sufficient to disregard the study because the concentration of 5.63 mg/L was in reality reached and kept constant throughout the exposure period. RAC also notes that the dense aerosol might preclude the assessment of the clinical signs but that these might have occurred. Another factor to be taken into consideration is that the key study proposed by the DS was performed with d-trans-tetramethrin, which contains around double the concentration of the isomer with highest neurotoxic potency compared to tetramethrin. RAC is also 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 tetramethrin for **acute inhalation toxicity.**

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

Summary of the Dossier Submitter's proposal

The DS proposed classification of 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 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 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 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 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 tetramethrin as **STOT SE Category 2 (H371: May cause damage to nervous system by inhalation route).**

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

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

- Two independent OECD TG 404 studies with New Zealand White (NZW) rabbits showed that 500 mg (4 hours of exposure) of tetramethrin did not 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 did not 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.

Assessment and comparison with the classification criteria

The table below summarises the available skin corrosion/irritation studies 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	500 mg, moistened with corn oil	0.5, 24, 48, 72 h:	Nakanishi, 1990

	NZW 3 M + 3 F	4h	No erythema, oedema or any other effects on skin observed Reversibility: N/A	

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 tetramethrin for **skin corrosion/irritation is warranted.**

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification of 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.

Assessment and comparison with the classification criteria

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

Table: Summary of the animal study on serious eye damage/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 405	Rabbit NZW 3 M	53 mg (equivalent to 0.1 mL, test substance mixed 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 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	Nakanishi, 1990

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

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 tetramethrin for **serious eye damage/irritation.**

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	Pyrethroids, including tetramethrin	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	One single case of a 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 the 1st): Tetramethrin powder diluted 1/10 in lactose powder	Skin prick testing to tetramethrin: negative Challenge-provoked reactions: Reduced respiratory function (reduced forced expiratory volume in 1 second; asthma) Patient treated with beta-agonist when required	Vandenplas *et al.*, 2000

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 tetramethrin and concluded on **no classification for respiratory sensitisation.**

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.

Assessment and comparison with the classification criteria

The table below summarises the Buehler & Magnusson and Kligman studies with <u>tetramethrin</u>.

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 Positive control: 5 M + 5 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

	Treatment group: 10 M + 10 F			
Buehler Similar to OECD TG 406 (with 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 Magnusson & Kligman protocol	Guinea pig Hartley 7 M Positive control: 5 M	1 % solution in corn oil 10 intracutaneous injections over 23 days Challenge 14 days later	No animals sensitised to tetramethrin (0/7) Positive control DNCB: 5/5	Okuno *et al*., 1976

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 tetramethrin.**

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.

Assessment and comparison with the classification criteria

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 16 M + 16 F per dose group Oral (dietary) 0, 500, 1500, 5000 ppm (0, 30, 95, 325 mg/kg bw/d) 6 months	5000 ppm: 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**	Suzuki and Okuno, 1977
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	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)	Walker, 1996

	(0, 8/9, 36/36, 147/157, 286/325 mg/kg bw/d (M/F)) 1 year	**NOAEL: 1200 ppm**	
OECD TG 413 GLP	Rat Crj:CD(SD) 10 M + 10 F per dose group Inhalative (whole body) 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	≥ 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 (M)), dark-red discoloration of liver, soft and large liver, hepatocellular hypertrophy, hyaline droplets in renal tubules (M) 0.824 mg/L: 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**	Kawaguchi, 1991

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	**Rat, 28 days, oral**	**290 (M)/295 (F)**	**30 ≤ C ≤ 300**

chemistry changes			
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).

	SEX	**Dose groups**			
		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 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 guideline concentration.

RAC noted that the severe effects in liver always appeared above the maximum 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 tetramethrin **does not meet the criteria for classification for STOT RE.**

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 tetramethrin for germ cell mutagenicity.

Comments received during public consultation

One MSCA supported the no classification proposal.

Assessment and comparison with the classification criteria

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 Non-GLP	*Salmonella. typhimurium:* TA1535, TA1537, TA97, TA98 and TA100 *Escherichia coli:* WP2uvrA	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)	Kogiso and Yoshitake, 1987
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	CHO cells	-S9: 15.1-80.3 µg/mL	+	-	Cytotoxicity:	Murli, 1989

Chromosomal aberration study		+ S9: 50.2-151 µg/mL			≥ 20.1 µg/mL (-S9, 20 h) ≥ 75.3 µg/mL (+S9, 20 h) ≥ 101 µg/mL (+S9, 30h)	
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: +S9: 75 µg/mL (Experiment II, 3 h) -S9: ≥ 50 µg/mL (Experiment II, 24 h)	Cinelli, 2004
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

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.

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 N°/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

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.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The DS proposed classification and labelling of 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 Satellite group: 40 M + 40 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
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	Non-neoplastic effects: ≥ 3000 ppm: Cytoplasmic vacuolation of midzonal hepatocytes (M), testes enlarged (M), reduced bw and food consumption Neoplastic effects: ≥ 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	Rat	0, 200, 1000, 5000 ppm (CR CD: 0, 7.5, 35, 180 mg/kg bw/d, Long Evans	Non-neoplastic effects: 5000 ppm: Reduced bw gain, slight increase in	Pence, 1981

Non-GLP Oral (dietary)	CR CD and Long Evans (LE) Hooded 50 M per strain/dose group	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	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	

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.

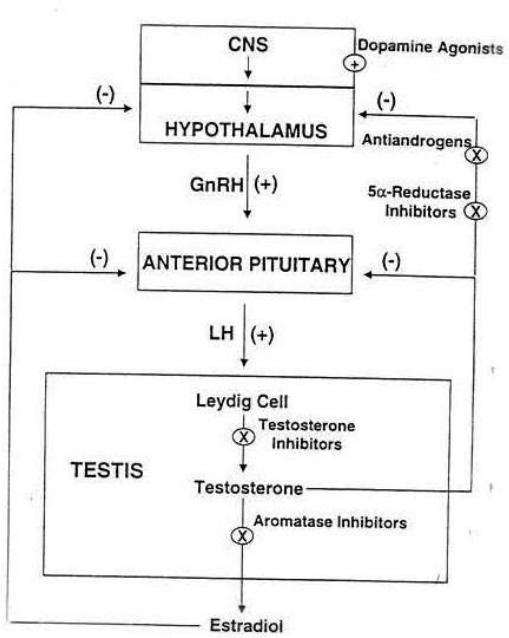
The mouse study showed neither non-neoplastic nor neoplastic lesions. However, both rat studies showed consistent results. The Rutter (1974) rat study showed testicular tumours already at the lowest dose (42 mg/kg bw/d), but achieved a statistically significant incidence starting at the medium dose (125 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.

<u>Gonadotropin-releasing hormone (GnRH) agonism</u>

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 60 000-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 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 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 tetramethrin for **Carcinogenicity Category 2 (H351: Suspected of causing cancer).**

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification or labelling of 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 classification proposal.

Assessment and comparison with the classification criteria

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 N°/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	Rat Slc: SD (SPF)	100, 300 and 1000 mg/kg bw/d	**Dams:** 300 and 1000 mg/kg bw/d: slight liver swelling and statistically sign. increased absolute	Sato *et al.*, 1980

Non-GLP	Male (for mating) and 11-13 (pretest)/20 (main study) females	Gavage Day 7 of pregnancy to day 21 of lactation	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**	
Pre-guideline Non-GLP One-generation reproduction toxicity 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

Development

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

Teratogenicity study in the rat OECD TG 414 GLP	Rat Crl:COBS VAF CD(SD)BR 25 females per dose group	0, 150, 500, 1000 mg/kg bw/d Gavage Day 6-15 of gestation	No treatment-related effects **NOAEL maternal/developmental: 1000 mg/kg bw/d**	Robinson *et al*., 1991
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 9 females per dose group	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, 1978

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 tetramethrin is warranted.

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.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The DS proposed 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 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 tetramethrin was investigated by two key studies (Sumitomo, 2002 and Endura, 2002) which were conducted according to OECD TG 301 F investigating ready biodegradability by measurement of biochemical oxygen demand. According to the test results, tetramethrin degraded 23% and 24% within 28 days respectively (Sumitomo, 2002 and Endura, 2002). Therefore, based on the CLP criteria, the DS concluded that tetramethrin can be considered as not readily biodegradable.

The soil and sediment degradation of tetramethrin was investigated in two laboratory studies according to OECD TG 307 and US-EPA 162-1, respectively. Although the results of the simulation studies demonstrated a rapid to moderate primary degradation, the ultimate degradation was considered as 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. Hence, tetramethrin cannot be considered as readily degradable, according to CLP criteria
A preliminary study on hydrolysis of tetramethrin was conducted according to OECD TG 111. The degradation of tetramethrin at 50°C was about 34% at pH 4.0 after 120 hours and 50% at pH 7.0 after 2.4 hours. At pH 9.0 the hydrolysis was fast and the test item was not detected in the sample solutions.

An 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. It was considered that the data is sufficient to characterise the route of degradation and the major degradation products of cis-tetramethrin, as water is a non-chiral solvent and (1RS)-trans-tetramethrin is hydrolytically cleaved at the imide and carboxylate ester moiety representing non-chiral centres. Hence, the results can be used to read across to tetramethrin. 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% and to 80.6% at pH 7 and pH 9 respectively. THPA increased to 66.07% at pH 5, 95.78% at pH 7, and 68.07% at pH 9. 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.

Photolysis in water. A preliminary study according to US EPA guideline OPPTS 835.2210 was conducted in sterile pH 5 buffer for (1R)-cis and (1R)-trans-tetramethrin. The definitive study

was conducted with (1R)-trans-tetramethrin, as the preliminary study showed a similar degradation pattern for both stereoisomers. Additionally, enantiomerism is not expected to affect significantly the aqueous photolysis under environmental conditions thus, the data are considered sufficient to characterise the route and kinetic of photodegradation and the major degradation products of the S-isomer. Hence, the results can be used to read across to tetramethrin.

(1R)-trans-tetramethrin underwent 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 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.

(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 significantly more rapidly when exposed to light, its hydrolytic degradation without irradiation had no substantial 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 827 L/$kg_{wetfish}$ (OECD TG 305) is available only for (1R)-trans-tetramethrin, information on the cis-isomers is lacking (Sumitomo, 1994). Estimation on aquatic bioconcentration is provided by Sumitomo (2006) with measured Log Kow = 4.58 and Endura (2003) with measured Log Kow ≥ 4.09. According to the standard equation of TGD Risk Assessment, estimated BCF for fish respectively was 1560 L/$kg_{wet\ fish}$, based on the Log Kow = 4.58, and > 598 L/$kg_{wet\ fish}$ based on the Log Kow ≥ 4.09.

With a maximum estimated BCF_{fish} of 1560 L/$kg_{wet\ fish}$ for tetramethrin, supported by a measured BCF_{fish} value of 827 L/$kg_{wet\ fish}$ for the trans-isomers, the DS considered tetramethrin as bioaccumulative according to CLP criteria.

Aquatic Toxicity

The ecotoxicological tests results for tetramethrin are summarised in the following table and sections.

Test organism / guideline, test method	**Short-term result (endpoint)**	**Long-term result (endpoint)**	**Reference**
Bluegill fish (*Lepomis macrochirus*) / EPA OPP 72-1	96-h LC_{50} = 0.016 mg/L (measured concentrations)	-	Bowman, 1990a
Rainbow trout (*Oncorhynchus mykiss*) / EPA OPP 72-1	96-h LC_{50} = 0,0037 mg/L (measured concentrations)	-	Bowman, 1990b
Zebra fish (*Danio rerio*) / OECD TG 203	96-h LC_{50} = 0.033 mg/L (measured concentrations)	-	Seyfried, 2002a

Test organism / guideline, test method	Short-term result (endpoint)	Long-term result (endpoint)	Reference
Zebra fish (*Danio rerio*) / OECD TG 203	96-h LC_{50} = 0.0349 mg/L (measured concentrations)	-	Croce, 2006
Daphnia magna / EPA OPP 72-2	48-h EC_{50} = 0.11 mg/L (measured concentrations)	-	Blasberg, 1993
Daphnia magna / OECD TG 202	48-h EC_{50} = 0.16 mg/L (measured concentrations)	-	Seyfried, 2002b
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 (mean measured concentrations)	Hoberg, 2002
Scenedesmus subspicatus / OECD TG 201	72-h E_rC_{50} = > 0.33 mg/L (measured concentrations)	72-h NOE_rC = 0.33 mg/L (mean measured concentrations)	Seyfried, 2002c

The DS identified fish as the most sensitive trophic group in acute aquatic toxicity studies and based the aquatic acute classification on the 96-h LC_{50} of 0.0037 mg/L (mortality) for *Oncorhynchus mykiss*.

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 the tetramethrin is considered to be not readily biodegradable and to have a potential to bioaccumulate, the DS based aquatic chronic classification on the 96-h LC_{50} of 0.0037 mg/L (mortality) for *Oncorhynchus mykiss*.

Comments received during public consultation

Two MSCA submitted comments and agreed with the proposed classification of 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 tetramethrin is not readily biodegradable based on 23% 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 tetramethrin is 4.58, which is above the CLP Log Kow trigger value of ≥ 4. The measured BCF_{fish} value is 827 $L/kg_{wet\ fish}$ for the tetramethrin trans-isomers, which is above the CLP BCF trigger value of 500. This BCF_{fish} value (827 $L/kg_{wet\ fish}$) related to total radioactive residues; whole fish and lipid content of 5% was obtained as a worst case in the environmental risk and hazard assessment. The estimated (from Log Kow) BCF_{fish} was 1560 $L/kg_{wet\ fish}$.

Based on the measured BCF value of 827 L/$kg_{wet\ fish}$, RAC agrees with the DS's conclusion that the substance has a potential bioaccumulate and should be considered as bioaccumulative.

Aquatic Toxicity

Reliable acute aquatic toxicity studies are available for fish, aquatic invertebrates and algae. 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 notes, 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 became in the future.

Acute toxicity

RAC agrees with the DS that the lowest acute toxicity for tetramethrin was observed for Rainbow trout (*Oncorhynchus mykiss*) with an acute 96 hours LC_{50} of 0.0037 mg/L (mean measured concentrations). The corresponding M-factor is 100.

Chronic toxicity

The lowest mean measured NOE_rC 0.25 mg/L was observed for the algae *Selenastrum capricornutum*. As no chronic data is available for the 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

Tetramethrin is considered not rapidly degradable and bioaccumulative. In agreement with the DS, RAC is of the opinion that 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**.

Additional references

Factsheets from the (eco)toxicological risk assessment strategy of the National Institute for Public Health and the Environment Part IV. RIVM report 601516012/2004. Available at: http://www.rivm.nl/dsresource?objectid=rivmp:15807&type=org&disposition=inline&ns_nc=1

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ANNEXES:

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

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