**Committee for Risk Assessment**

**RAC**

Annex 2

**Response to comments document (RCOM)**

to the 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**

**ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 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**

## COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the public consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the public consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties.

ECHA accepts no responsibility or liability for the content of this table.

**Substance 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**
**Dossier submitter: Germany**

### GENERAL COMMENTS

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 04.02.2016 | France | | MemberState | 1 |
| Comment received | | | | |
| We agree with the classification proposal. | | | | |
| Dossier Submitter's Response | | | | |
| Thank you. | | | | |
| RAC's response | | | | |
| Noted. Thank you. | | | | |

### CARCINOGENICITY

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 05.02.2016 | Italy | Endura S.p.A. | Company-Manufacturer | 2 |
| Comment received | | | | |
| Endura's Proposal: No classification<br>Based on available data, there is a weight of evidence that the mechanisms by which tetramethrin causes LCTs in rats is not relevant for humans. A separate attachment is provided.<br><br>ECHA note: The following attachment was submitted with the comment above:<br>*TTM CLH Endura Carcinogenicity 160205* | | | | |
| Dossier Submitter's Response | | | | |
| The attachment was noted. However, the available mechanistic information is still regarded insufficient to demonstrate that the observation in rats is not relevant to humans. | | | | |

In both rodents and humans, luteinizing hormone (LH) stimulates Leydig cells to produce testosterone. Rat Leydig cells have approx. 20,000 LH receptors compared to an estimated 1500 LH receptors in human Leydig cells (Huhtaniemi, 1983). This is thought to confer a higher sensitivity to changes in LH levels to rat Leydig cells compared to the human Leydig cell. In order to decide on whether these quantitiative species differences may justify the conclusion on non-relevance of Leydig cell tumors (LCT) induction by tetramethrin in the rat, mechanistic information would be required. This includes parameters such as:

- histopathology of the testis, epididymis, prostate, seminal vesicles with coagulating glands, pituitary gland, and liver (processed by standard histologic procedures, stained with hematoxylin and eosin),
- LC proliferation via 5-bromo-2'-deoxyuridine (BrdU) (for identification of BrdU incorporation into nuclear DNA as a surrogate marker of cell proliferation),
- testis and liver gene expression (for example in liver samples: *Cyp2b1* "CAR response gene"; *Cyp3a23*/*3a1* "PXR response gene" etc. , in testis samples:*Cyp4a1*/*4a22* "PPAR-α response gene" etc.)
- *in vitro* metabolism of testosterone by liver microsomes,
- quantification of serum hormones (i.e. concentration of luteinizing hormone (LH), testosterone, follicle stimulating hormone (FSH), estradiol) and testosterone metabolites after treatment with different doses of tetramethrin (comparable to the dosage in the long-term study in rats).

At least nine known modes-of-action for Leydig cell tumor induction in rats, which fall into three categories of human relevance (i.e., relevant, low relevance, no relevance) were discussed in the literature (Cook et al., 1999). These include:

Relevant to Humans

(1) Mutagenicity

Low Relevance to Humans

(2) Androgen receptor antagonism
(3) Estrogen receptor agonism/antagonism
(4) 5-alpha-reductase inhibition
(5) Aromatase inhibition
(6) Reduced testosterone biosynthesis
(7) Increased testosterone metabolism

No Relevance to Humans

(8) GnRH (LHRH) agonism
(9) Dopamine agonism/enhancement

With respect to the above mentioned parameters, no additional investigations were performed / submitted. The mode of action by which tetramethrin leads to Leydig cell adenoma has not been sufficiently clarified, the relevance to humans is unclear and a classification for carcinogenicity, category 2, is proposed.

RAC's response

RAC agrees and supports the DS's response.

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 05.02.2016 | United Kingdom | Sumitomo Chemical (UK) Plc | Company-Manufacturer | 3 |

Comment received

Tetramethrin: Opinion on the significance of testicular interstitial (Leydig) cell tumours in Two-year rat chronic/carcinogenicity dietary studies

Summary:
Testicular interstitial (Leydig) cell tumours are a frequently occurring, largely species-specific tumour in rats, often associated mechanistically with mild hormonal imbalances. Such tumours are not considered to be an appropriate model for assessing the potential risk to human males of developing such a rare testicular tumour. Indeed, the International Programme on Chemical Safety (IPCS), Environmental Health Criteria 98, on Tetramethrin, confirms this opinion and states in their Appraisal on page 23 that: 'It can be concluded that the tumorigenic effect, if real, is most unlikely to be relevant to human exposure.'
Current understanding of the lesion indicates that the mode of action in the rat typically involves a response of the testicular interstitial cells to sustained stimulation by luteinizing hormone (LH) which, as with many endocrine tissues, has been demonstrated to result in the induction of hyperplasia and, subsequently over time, benign tumours. Studies utilizing a range of substances have demonstrated that this is a species-specific sensitivity of the rat, compared with the mouse, due to a difference between the Leydig cell responses in the two species.
On the basis of the large and diverse number of substances that induce testicular interstitial cell tumours in rats but not in mice, it is clear that rats and mice differ markedly in their sensitivity to the induction of interstitial cell tumours. In terms of the relevance of such tumours to man, it has been noted that rat interstitial cells in vivo have been reported to exhibit much greater proliferative capacity than human testicular interstitial cells. Therefore, when all the available data are considered collectively, it leads to a weight of evidence that the induction of testicular interstitial cell tumours in rats following exposure to Tetramethrin represents a species-specific sensitivity which is unlikely to represent a hazard for man. Pragmatically this is supported by the observation that by simply including 20% lactose in the diet of rats, interstitial cell tumours are induced, while the widespread use and consumption of lactose by the human population has not been associated with any such observation in man. A range of other substances, including agrochemicals, metals, pharmaceuticals and plasticisers have been shown to have this effect in rats with no evidence for such testicular effects in man.
Overall, based on a large weight of evidence, and acknowledging that human testicular interstitial cells are refractory to the tumorigenic effects of many substances, the data strongly support the conclusion that testicular tumour formation by Tetramethrin in rats is not a relevant finding for humans. Therefore, it can be reasonably concluded that, based on current criteria and available evidence, Tetramethrin should remain Not Classified for carcinogenicity.

ECHA note - The following attachment was submitted with the comment above:
*20160205 CLH Proposal Tetramethrin - Comments from Sumitomo Chemical*

Dossier Submitter's Response

We disagree with the conclusion of Sumitomo.

| |
|---|
| Although the mechanism discussed in the comment is widely regarded as not relevant to humans due to quantitative interspecies differences, it remains unclear, whether the LCT induced by tetramethrin in rats is due to this specific mechanism. Further mode of actions for LCT induction exist. Please also refer to our response to the comment no. 2 by Endura concerning carcinogenicity. |
| RAC's response |
| RAC agrees and supports the DS's response. |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 05.02.2016 | Sweden | | MemberState | 4 |
| Comment received | | | | |
| The Swedish CA support classification of (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 (CAS No. 7696-12-0) in Carc. 2. SE agree with the rationale for classification into the proposed hazard class and category.<br><br>Oral exposure to tetramethrin during 104 weeks resulted in an increased incidence in interstitial adenomas of the tesitis (Leydig cell tumours) in rats established in two independent studies, but not in mice where no induction of neoplastic or non-neoplastic effects was observed in the one study available. Findings of Leydig cell tumours in rats or mice would normally lead to classification for carcinogenicity, and substances that have been shown to induce such tumours should be classified in Carc. 2, unless the mechanism can be proven not to be relevant to humans. Since, in this case, the underlying mechanism is not known, its relevance to humans cannot be ruled out. Accordingly, we support that tetramethrin should be classified in Carc. 2 (H351). | | | | |
| Dossier Submitter's Response | | | | |
| Not required. | | | | |
| RAC's response | | | | |
| Noted. Thank you. | | | | |

**OTHER HAZARDS AND ENDPOINTS – Acute Toxicity**

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 05.02.2016 | Italy | Endura S.p.A. | Company-Manufacturer | 5 |
| Comment received | | | | |
| Endura's proposal: No classification for acute inhalation toxicity<br>The study that would support not to classify tetramethrin is valid. Instead, the study that led to the classification proposal should be considered not suitable for classification. A separate attachment is provided.<br><br>ECHA note: The following attachment was submitted with the comment above:<br>*TTM CLH Endura Inhalation 160205* | | | | |
| Dossier Submitter's Response | | | | |
| The dossier submitter has evaluated the acute inhalation toxicity study no 4414/05 that was considered by Endura as "valid". The evaluation of the dossier submitter has recently been confirmed by several MS authorities during the biocides review programme. According to the study report, only slight lacrimation and nasal discharge were observed | | | | |

in all rats on day 1 at the tested dose (analytical conc. 5.63 mg/L). During exposure, clinical observation was not possible. This is in sharp contrast to the other acute inhalation study submitted by Sumitomo, which reported a decrease in spontaneous activity, hyperexcitability, hyperpnea and irregular respiration, muscular fibrillation, urinary incontinence and salivation in a dose dependent manner from concentrations of 0.13 mg/L. Also, there was 1/10 lethality for exposed females at 1.18 mg/L in this study. In an subacute inhalation study, equivalent acute effects (bradypnoe, irregular respiration, salivation, hyperexcitability) were reported as acute effects observed daily during exposure at concentration of 0.09 mg/L. The same acute effects were noted in a subchronic inhalation study at concentrations of 0.13 and 0.82 mg/L. Thus, the study considered by Endura as valid is the only one in a series of studies, that did not show the acute neurotoxic effects attributable to pyrethroid intoxiciation.

We agree that the acute inhalation toxicity study submitted by Sumitomo also shows deficiencies, with the most severe deficiency relating to the selection of the high dose. At the highest tested dose of 1.18 mg/L, 10% lethality in females was reported. Reliable data from other acute inhalation toxicity studies was not submitted in the biocides assessment.

RAC's response

RAC understands that, with this new information, there are no reasons to disregard this study. Therefore, it will be taken into consideration in the assessment of acute toxicity by inhalation route.

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 05.02.2016 | Finland | | MemberState | 6 |

Comment received

German CA proposes Tetramethrin to be classified for Acute tox 4; H332 and STOT SE 2; H371.

The proposed classification, Acute tox. 4; H332, seems to be primarily based on one acute inhalation study in rat by Suzuki et al. 1981 (pre-guideline, non-GLP study). The duration of exposure in the study was shorter (only 3 hours instead of 4) than recommended according to TG 403. At the highest concentration level 1.18 mg/L one female rat died (out of 20 animals in total). Signs of neurotoxicity were reported at the concentrations of 0.13 mg/L and above. Information about the cause and time of death is not available in the CLH report. The Finnish CA considers that more information is needed to support the proposed classification.

The proposed classification, Acute tox. 4; H332, is based on one death seen at the highest concentration level. Exact determination of the LC50 is not possible, due to only one death. The DS has estimated the LC50 value to be above 1.18 mg/L. The selection of the upper concentration value for LC50 is based on assumption that `it cannot be ruled out that the LC50 is ≤ 5 mg/L`. The LC50 value used for the classification is highly uncertain. The Finnish CA does not consider the classification proposal sufficiently justified.

The proposed classification, STOT-SE 2; H371 for inhalation, is based on signs of neurotoxicity seen in the acute inhalation study and 90 –days repeated dose toxicity study (Suzuki et al. 1981, Kawaguchi et al. 1991). It is difficult to evaluate significance and relevance of observed effects because of poor reporting in the studies. For instance,

the number of affected animals and severity of effects were not clearly documented. The opinion of the Finnish CA is that the proposed classification STOT-SE; H371 needs further justification. In the evaluation of the data it should be considered that the substance is a pyrethroid and belongs to a class of chemicals having potential to induce neurotoxic effects.

Based upon the reported data in the CLH report, neurotoxic effects may have been responsible for the mortality at the highest concentration level in the Suzuki et al. 1981 study. Care must be taken not to classify for STOT-SE and Acute Tox. using the same effect. This would lead to double classification.

Dossier Submitter's Response

With regard to the acute inhalation toxicity study (Suzuki et al., 1981) at a dose of ≥ 0.13 mg/L air toxic signs began to appear 15-30 min after initiation of exposure. The incidence was not clearly reported. Signs disappeared 1-2 hours after the end of exposure. No further information was given in the report with respect to clinical signs and mortality. However, considering the neurotoxic MoA of pyrethroids and the neurotoxic signs, lethality was presumably due to neurotoxicity.

In the subacute inhalation toxicity study at a dose of ≥ 0.087 mg/L air toxic signs were noted every day during the exposure period. They were not cumulative.

For the subchronic study, clinical signs on day 1 (during and after exposure) are summarised in the tables below. Clear signs of neurotoxicity can be observed after single exposure to 0.824 mg/L. This would correspond to the guidance range for STOT SE1.

We agree that double classification should be avoided. STOT SE should be applied, when no Acute Tox is required. This will in particular be the case for mixtures containing tetramethrin as the result of the different rules for classification of mixtures. For STOT SE2, the generic concentration limit is 10%, while the GCLs for STOT SE1 are 1-10% (Cat2) and 10% (Cat1). This is lower than the limits that would result for Acute Tox classification of a mixture containing no other ingredient classiefied as acutely toxic by inhalation than Tetramethrin.

Table 8 Clinical signs

Three-month inhalation toricity study of Neo-Pynamin in rats

| Sex | Group (mg/m³) | Clinical signs | Day 1 B | Day 1 C |
|---|---|---|---|---|
| Male | Vehicle control | No. of Animals | 10 | 10 |
| | | No abnormal sign | 5 | 10 |
| | | Bradypnea | 0 | 0 |
| | | Irregular respiration | 5 | 0 |
| | | Decrease of spontaneous activity | 0 | 0 |
| | | Rough coat | 0 | 0 |
| | | Loss of hair | 0 | 0 |
| | | Scab | 0 | 0 |
| | | Wet fur | 0 | 0 |
| | Air control | No. of Animals | 10 | 10 |
| | | No abnormal sign | 10 | 10 |
| | | Loss of hair | 0 | 0 |
| | 20.3 | No. of Animals | 10 | 10 |
| | | No abnormal sign | 4 | 10 |
| | | Irregular respiration | 6 | 0 |
| | | Decrease of spontaneous activity | 0 | 0 |
| | | Rough coat | 0 | 0 |
| | | Loss of hair | 0 | 0 |
| | | Scab | 0 | 0 |
| | | Erosion | 0 | 0 |
| | | Wet fur | 0 | 0 |
| | 134 | No. of Animals | 10 | 10 |
| | | No abnormal sign | 2 | 10 |
| | | Bradypnea | 0 | 0 |
| | | Irregular respiration | 8 | 0 |
| | | Decrease of spontaneous activity | 3 | 0 |
| | | Rough coat | 0 | 0 |
| | | Loss of hair | 0 | 0 |
| | | Scab | 0 | 0 |
| | | Swelling | 0 | 0 |
| | | Wet fur | 0 | 0 |
| | | Red tear | 0 | 0 |

A : Before exposure. B : During exposure. C : After exposure.

| Sex | Group (mg/m³) | Clinical signs | Day 1 B | Day 1 C |
|---|---|---|---|---|
| Male | 824 | No. of Animals | 10 | 10 |
| | | No abnormal sign | 0 | 8 |
| | | Bradypnea | 10 | 0 |
| | | Irregular respiration | 10 | 1 |
| | | Decrease of spontaneous activity | 10 | 0 |
| | | Rough coat | 0 | 0 |
| | | Loss of hair | 0 | 0 |
| | | Scab | 0 | 0 |
| | | Wet fur | 0 | 0 |
| | | Conjunctival discharge | 0 | 0 |
| | | Red tear | 2 | 0 |
| | | Nasal discharge | 3 | 0 |
| | | Dark red substance attaching around snout | 0 | 0 |
| | | Salivation | 10 | 1 |
| | | Urinary incontinence | 0 | 0 |

A : Before exposure. B : During exposure. C : After exposure.

Table 8 Clinical signs

Three-month inhalation toxicity study of Neo-Pynamin in rats

| Sex | Group (mg/m³) | Clinical signs | Day 1 B | Day 1 C |
|---|---|---|---|---|
| Female | Vehicle control | No. of Animals | 10 | 10 |
| | | No abnormal sign | 10 | 10 |
| | | Bradypnea | 0 | 0 |
| | | Irregular respiration | 0 | 0 |
| | | Decrease of spontaneous activity | 0 | 0 |
| | | Rough coat | 0 | 0 |
| | | Loss of hair | 0 | 0 |
| | | Scab | 0 | 0 |
| | | Wet fur | 0 | 0 |
| | Air control | No. of Animals | 10 | 10 |
| | | No abnormal sign | 10 | 10 |
| | | Loss of hair | 0 | 0 |
| | 20.3 | No. of Animals | 10 | 10 |
| | | No abnormal sign | 9 | 10 |
| | | Irregular respiration | 1 | 0 |
| | | Decrease of spontaneous activity | 0 | 0 |
| | | Rough coat | 0 | 0 |
| | | Loss of hair | 0 | 0 |
| | | Scab | 0 | 0 |
| | | Erosion | 0 | 0 |
| | | Wet fur | 0 | 0 |
| | 134 | No. of Animals | 10 | 10 |
| | | No abnormal sign | 5 | 10 |
| | | Bradypnea | 0 | 0 |
| | | Irregular respiration | 5 | 0 |
| | | Decrease of spontaneous activity | 0 | 0 |
| | | Rough coat | 0 | 0 |
| | | Loss of hair | 0 | 0 |
| | | Scab | 0 | 0 |
| | | Swelling | 0 | 0 |
| | | Wet fur | 0 | 0 |
| | | Red tear | 0 | 0 |

A : Before exposure. B : During exposure. C : After exposure.

Table 8 Clinical signs

Three-month inhalation toxicity study of Neo-Pynamin in rats

| Sex | Group (mg/m³) | Clinical signs | Day 1 B | Day 1 C |
|---|---|---|---|---|
| Female | 824 | No. of Animals | 10 | 10 |
| | | No abnormal sign | 0 | 9 |
| | | Bradypnea | 10 | 0 |
| | | Irregular respiration | 10 | 0 |
| | | Decrease of spontaneous activity | 9 | 0 |
| | | Rough coat | 0 | 0 |
| | | Loss of hair | 0 | 0 |
| | | Scab | 0 | 0 |
| | | Wet fur | 0 | 0 |
| | | Conjunctival discharge | 0 | 0 |
| | | Red tear | 3 | 0 |
| | | Nasal discharge | 5 | 0 |
| | | Dark red substance attaching around snout | 0 | 0 |
| | | Salivation | 10 | 0 |
| | | Urinary incontinence | 0 | 1 |

A : Before exposure. B : During exposure. C : After exposure.

RAC's response

RAC agrees with the comment about the acute toxicity. It is not possible, with the available information, to set an LC50 by inhalation route with enough accuracy to conclude on classification.

However, RAC agrees with the DS that the severity of the neurotoxicity reported at 0.824 mg/L is enough for classification and that the fact that the effects were reversible and non-cumulative points to single acute toxicity effects. Therefore, RAC supports the classification as STOT SE Category 2.

## OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 05.02.2016 | Belgium | | MemberState | 7 |
| Comment received | | | | |
| Based on the result of the acute aquatic toxicity test on the most sensitive species (fish - Oncorhynchus mykiss- with a 96hLC50=0.0037 mg/l), tetramethrin should be classified as Aquatic Acute 1, H400. In view of this classification and the toxicity band for acute toxicity between 0.001mg/l and 0.01 mg/l, a M-factor for acute toxicity of 100 should be assigned.<br><br>Results of chronic tests are only available for algae. Therefore the surrogate approach should also be explored and the most stringent outcome should be used for classification :<br>chronic classification should be based here on the LC50.<br><br>Classification based on NOEC algae : 72hNOErC=0.25mg/l(mm) results in a classification as Aquatic chronic 2, H410.<br>Tetramethrin is not rapidly degradable and based on the lowest LC50 (fish : 0.0037mg/l) it should be classified as Aquatic chronic 1, H410. A M-factor for acute toxicity of 100 should be attributed (chronic toxicity band between 0.001mg/l and 0.01 mg/l).<br><br>In conclusion: We support the proposed environmental classification of BAuA. | | | | |
| Dossier Submitter's Response | | | | |
| Thank you. | | | | |
| RAC's response | | | | |
| Noted. | | | | |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 05.02.2016 | Sweden | | MemberState | 8 |
| Comment received | | | | |
| The Swedish CA support the classification of Tetramethrin in Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410) as specified in the proposal. This conclusion is based on the effect of the most sensitive fish Oncorhynchus mykiss and that the substance is not rapidly degradable and has a high bioaccumulation potential.<br>The SE CA do agree with the rationale for the setting of M-factors of 100 for both acute and chronic toxicity for the aquatic organisms. | | | | |
| Dossier Submitter's Response | | | | |
| Thank you. | | | | |
| RAC's response | | | | |
| Noted. | | | | |

## NON-CONFIDENTIAL ATTACHMENTS:

1. TTM CLH Endura Carcinogenicity 160205. Submitted on 05/02/2016. [Please refer to comment No 2]
2. TTM CLH Endura Inhalation 160205. Submitted on 05/02/2016. [Please refer to comment No 5]

## CONFIDENTIAL ATTACHMENTS:

1. *20160205 CLH Proposal Tetramethrin - Comments from Sumitomo Chemical*. Submitted on 05/02/2016. [Please refer to comment No 3]