

**Agency technical report on the classification  
and labelling of:**

**Melaleuca alternifolia, ext. [1]**

***Melaleuca alternifolia*, essential oil; tea tree oil  
[2]**

EC Number: 285-377-1 [1], – [2]

CAS Number: 85085-48-9 [1], 68647-73-4 [2]

**August 2024**



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## Brief summary

The conclusion of the Agency technical report is that Melaleuca alternifolia, ext. [1] Melaleuca alternifolia, essential oil; tea tree oil [2] meets the classification criteria for:

Flam Liq. 3; H226 (Flammable liquid or vapour)

Acute Tox. 4; H302 (Harmful if swallowed) with an ATE of 1050 mg/kg bw

Acute Tox. 4; H332 (Harmful if inhaled) with an ATE of 3.60 mg/L (dusts or mists)

Skin. Irrit. 2; H315 (Causes skin irritation)

Skin Sens. 1B; H317 (May cause an allergic skin reaction)

Repr. 1B; H360F (May damage fertility)

Asp. Tox. 1; H304 (May be fatal if swallowed and enters airways)

Aquatic Acute 1; H400 (Very toxic to aquatic life) with an Acute M-factor of 1

Aquatic Chronic 2; H411 (Toxic to aquatic life with long lasting effects)

### **Is this in agreement with the RAC opinion?      NO**

RAC concluded that Melaleuca alternifolia, ext. [1] Melaleuca alternifolia, essential oil; tea tree oil [2] warranted classification with STOT SE 3; H336 (May cause drowsiness or dizziness) and Repr. 2; H361d (Suspected of damaging the unborn child). The Agency does not agree that classification is warranted for these hazard classes.

At the time of publication, this mandatory classification and labelling (MCL) has not been agreed and/or adopted in Great Britain.

## Introduction

Under Article 37 of the GB CLP Regulation<sup>1</sup>, the Agency<sup>2</sup> is required to produce a technical report for each substance on which the Committee for Risk Assessment (RAC) of the European Chemicals Agency produces an opinion<sup>3</sup>.

This technical report documents an independent scientific assessment, conducted by HSE technical specialists with support from the Environment Agency for the environmental hazard classification of the classification and labelling of Melaleuca alternifolia, ext. [1] Melaleuca alternifolia, essential oil; tea tree oil [2].

**Table 1: Information considered in the scientific assessment**

Document	Included in assessment
EU CLH report	Yes
Annexes to the EU CLH report	Yes
RAC opinion	Yes
Background document	Yes
Information submitted during the EU public consultation process (RCOM table, including attachments)	Yes
RAC minority opinion(s)	Not applicable
Other information:	No

This information has been evaluated against the classification and labelling criteria set out in the GB CLP Regulation.

<sup>1</sup>The retained CLP Regulation (EU) No. 1272/2008 as amended for Great Britain

<sup>2</sup> HSE acting in its capacity as the GB CLP Agency

<sup>3</sup> Under Article 37(4) of Regulation (EU) No 1272/2008 on classification, labelling and packaging of substances and mixtures

## Overview of current and proposed classification and labelling

Table 2: Current and proposed classification and labelling

	Index No.	International Chemical Identification	EC No.	CAS No.	Classification		Labelling			Specific Concentration 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)		
<b>GB MCL List entry</b>	No current entry	No current entry									
<b>EU dossier submitter's proposal</b>	TBD	Melaleuca alternifolia, ext. [1] Melaleuca alternifolia, essential oil; tea tree oil [2]	285-377-1 [1] - [2]	85085-48-9 [1] 68647-73-4 [2]	Flam. Liq. 3 Acute Tox. 4 Acute Tox. 4 Asp. Tox. 1 Skin Irrit. 2 Skin Sens. 1B Repr. 2 Aquatic Acute 1 Aquatic Chronic 3	H226 H361f H332 H302 H304 H315 H317 H400 H412	GHS02 GHS08 GHS07 GHS09 Dgr	H226 H361f H332 H302 H304 H315 H317 H410		oral: ATE = 1049 mg/kg bw  Inhalation: ATE= 3.64 mg/L (dusts or mists)  M = 1	
<b>EU RAC opinion</b>	TBD	Melaleuca alternifolia, ext. [1] Melaleuca alternifolia, essential oil; tea tree oil [2]	285-377-1 [1] - [2]	85085-48-9 [1] 68647-73-4 [2]	Flam. Liq. 3 Acute Tox. 4 Acute Tox. 4 STOT SE 3 Asp. Tox. 1 Skin Irrit. 2 Skin Sens. 1B Repr. 1B Aquatic Acute 1 Aquatic Chronic 2	H226 H302 H332 H336 H304 H315 H317 H360Fd H400 H411	GHS02 GHS08 GHS07 GHS09 Dgr	H226 H302 H332 H336 H304 H315 H317 H360Fd H410		oral: ATE = 1050 mg/kg bw  Inhalation: ATE= 3.60 mg/L (dusts or mists)  M = 1	
<b>Agency technical report conclusion</b>	TBD	Melaleuca alternifolia, ext. [1] Melaleuca alternifolia,	285-377-1 [1] - [2]	85085-48-9 [1] 68647	Flam. Liq. 3 Acute Tox. 4 Acute Tox. 4 Asp. Tox. 1 Skin Irrit. 2	H226 H302 H332 H304 H315	GHS02 GHS08 GHS07 GHS09 Dgr	H226 H302 H332 H304 H315		oral: ATE = 1050 mg/kg bw	

	Index No.	International Chemical Identification	EC No.	CAS No.	Classification		Labelling			Specific Concentration 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)		
		essential oil; tea tree oil [2]		-73-4 [2]	Skin Sens. 1B Repr. 1B Aquatic Acute 1 Aquatic Chronic 2	H317 H360F H400 H411		H317 H360F H410		Inhalation: ATE= 3.60 mg/L (dusts or mists)  M = 1	
<b>Resulting MCL entry on GB MCL list</b>	TBD	Melaleuca alternifolia, ext. [1] Melaleuca alternifolia, essential oil; tea tree oil [2]	285-377-1 [1] - [2]	85085-48-9 [1] 68647-73-4 [2]	Flam. Liq. 3 Acute Tox. 4 Acute Tox. 4 Asp. Tox. 1 Skin Irrit. 2 Skin Sens. 1B Repr. 1B Aquatic Acute 1 Aquatic Chronic 2	H226 H302 H332 H304 H315 H317 H360F H400 H411	GHS02 GHS08 GHS07 GHS09 Dgr	H226 H302 H332 H304 H315 H317 H360F H410		oral: ATE = 1050 mg/kg bw  Inhalation: ATE= 3.60 mg/L (dusts or mists)  M = 1	

TBD: to be determined

## Background

Active substance in Plant Protection Products:

Active substance in Biocidal Products:

Chemical registered under REACH:

Tea Tree Oil (TTO) is a fungicide with applications including on grapes and tomatoes. It also has consumer users, such as in health care products (e.g. fragrances and as food flavours) and cosmetics for human and animal use (e.g. mouthwash, toothpaste, shampoo, deodorants, lotions and antifungal treatment) (ECHA, 2023).

TTO is a liquid and has a maximum vapour pressure of 2100 Pa at 25°C. The water solubility is 1420 mg/L. The Log Pow is 3.4-5.5 at 30°C (ECHA, 2023).

TTO is a UVCB substance (unknown or variable composition, complex reaction products or biological materials), and has a complex composition including various terpene hydrocarbons (monoterpenes, sesquiterpenes and their associated alcohols). The composition of TTO is outlined in the table below:

**Table 3: Example composition of TTO according to ISO 4730:2017, taken from page 4 of the RAC opinion (ECHA, 2023)**

Name	CAS No.	EC No.	Min. %	Max. %
Terpinen-4-ol	562-74-3	209-235-5	30	48
$\gamma$ -Terpinene	99-85-4	202-794-6	10	28
$\alpha$ -Terpinene	99-86-5	202-795-1	5	13
$\alpha$ -Terpineol	98-55-5	202-680-6	1.5	8
$\alpha$ -Terpinolene	586-62-9	209-578-0	1.5	5
$\alpha$ -Pinene	80-56-8	201-291-9	1	6
p-Cymene	99-87-6	202-796-7	0.5	8
1,8-Cineole (Eucalyptol)	470-82-6	207-431-5	trace	15
Limonene	138-86-3	205-341-0	0.5	1.5
Aromadendrene	489-39-4	207-694-6	0.5	3
$\delta$ -Cadinene	483-76-1	--	trace	3
Sabinene	3387-41-5	222-212-4	trace	3.5
Globulol	489-41-8	207-696-7	trace	1
Viridiflorol	552-02-3	209-003-3	trace	1
Ledene	21747-46-6	244-565-3	trace	3

During the first ECHA public consultation, comments were received noting that, as well as being used in pesticides/plant protection products (PPPs) there were a large number of other versions of this substance used in biocides and as ingredients in pharmaceuticals and cosmetics and registered under REACH. A variety of EC and CAS numbers for various grades of Tea Tree Oil, possibly derived using different extraction methods from the plant *Melaleuca alternifolia*, were identified from ECHA's Information on Chemicals database. It was questioned whether these were different enough to be considered distinct substances, or alternatively similar enough to be covered all under one harmonised classification.

In the response to comments document (RCOM, ECHA, 2023), the Dossier Submitter (DS) stated that the substance 'tea tree oil' has been commonly described by the following identifiers:

1. "Essential oils, *Melaleuca alternifolia*" CAS 68647-73-4 describing the pesticide active substance according to the Regulation (EC) 1107/2009 and
2. "*Melaleuca alternifolia*, ext.", EC 285-377-1, CAS 85085-48-9 describing the substance registered under REACH.

The DS concluded that the compositions and manufacturing processes reported in both cases correspond to the same substance. Therefore, the above identifiers were included in the proposed EU CLP Annex VI entry. Under EU REACH, ECHA has received other classification and labelling notifications describing similar substances under various names and some of these were given different EC list numbers. However, the information provided on the identity and composition of these substances is limited, therefore the DS proposed not to include these names in the CLH proposal. In conclusion, the active substance was identified with the names and identifiers: *Melaleuca alternifolia*, essential oil; tea tree oil; CAS 68647-73-4 and *Melaleuca alternifolia*, ext. CAS 85085-48-9; EC 285-377-1. The name 'extract of tea tree' was not considered appropriate by the DS to clearly identify the substance subject to this classification.

However, in a subsequent targeted public consultation in March 2023, the DS released a separate stand-alone version CLH report using the standard template (dated January 2022, so prior to the first report) with the title: 'International Chemical Identification: Extract from tea tree; Tea Tree Oil (TTO); Oil of *Melaleuca alternifolia* (terpinen-4-ol type)' - and using only EC No.: 285-377-1 and CAS No.: 68647-73-4. The main CLP-relevant text and conclusions were almost identical to the earlier version of the report.

**For the purposes of this GB MCL Technical Report, the 'substance' subject to this classification will simply be referred to as Tea Tree Oil (or TTO).**

### Toxicokinetics

RAC noted that there were no toxicokinetic data on TTO itself. However, based on the substance's constituents, RAC assumed that TTO is metabolised and excreted in animals within 2-3 days, mainly through the urine. RAC expected no bioaccumulation to occur.

# Scientific assessment of the physical, human health and environmental hazard classes

## Physical Hazards

### Classification agreed by RAC:

#### *Explosives*

A Differential Scanning Calorimetry (DSC) test was performed over the range 30- 400°C. No significant exothermic effects occurred during the test, and RAC hence concluded that TTO was very unlikely to undergo a thermally induced explosive reaction. Screening conducted by the DS did not reveal the presence of any chemical groups associated with explosive properties. RAC additionally noted that the oxygen balance of TTO's constituents is below the trigger of -200.

Overall, RAC concluded that classification for explosive properties was not warranted.

#### *Flammable liquids*

Testing in accordance with EC Method A.9 revealed the flash point of TTO to be 54-55°C at 100.7-102.1 kPa. This temperature is  $\geq 23^{\circ}\text{C}$  and  $\leq 60^{\circ}\text{C}$  and the pressure for determination is close to 101.3 kPa, therefore, RAC concluded that TTO should be classified as Flam. Liq. 3; H226 (Flammable liquid and vapour).

#### *Self-reactive substances*

No significant exothermic events occurred during DSC testing. The auto-ignition temperature of TTO was tested in two different studies according to EC Method A.15, and was found to be 252°C and 269°C. In addition, RAC noted that there were no chemical groups associated with explosivity and/or self-reactive properties.

RAC concluded that classification was not warranted for self-reactive substances.

### *Pyrophoric liquids*

Pyrophoric properties were tested as part of the auto flammability test (EC A.15). After 5 minutes, no ignition or charring of filter paper was observed. RAC noted that the United Nations Recommendations for the Transport of Dangerous Goods (UN RTDG) N.3 test is required to assess this endpoint under the CLP Regulation. Nonetheless, RAC noted that the screening procedure (based on experience in manufacturing/handling) could be used to conclude on the classification.

RAC concluded that classification as a pyrophoric liquid was not warranted.

### *Self-heating substances*

RAC noted that the auto-ignition temperatures of TTO could exclude the potential for the substance to self-heat. However, RAC noted that, according to the Guidance on the Application of the CLP Criteria, *'the phenomenon of self-heating applies mainly to solids'*, and *'substances with a low melting point e.g. <160°C should not be considered for classification, as the melting process is endothermic and the substance-air surface is drastically reduced'*. RAC considered that these points applied to TTO as it has a melting point of -22°C.

RAC concluded that classification was not warranted for self-heating substances.

### *Substances which in contact with water emit flammable gases*

An OECD TG 105 (water solubility study; 2007) was provided in the draft renewal assessment report (DRAR; the study was not referred to by the DS). RAC noted that no decomposition for any of TTO constituents occurred when they came into contact with water.

RAC concluded that classification was not warranted for this hazard class.

### *Oxidising liquids*

The DS presented four arguments to support not classifying for this hazard class, including the following:

- *'The ingredients do not contain any oxygen, or the oxygen is chemical bonded to carbon or hydrogen only'*.

The argument above was deemed sufficient to support no classification for this endpoint. Other arguments presented by the DS in the CLH report were not considered by RAC to be relevant for classification purposes.

RAC concluded that classification for oxidising liquids was not warranted.

#### *Corrosive to metals*

RAC noted that according to Section 2.16.4.1 of the Guidance on the Application of the CLP Criteria (ECHA, 2024) the following substances and mixtures should be considered for classification in this class:

- Substances and mixtures having acidic or basic functional groups
- Substances or mixtures containing halogen
- Substances able to form complexes with metals and mixtures containing such substances

RAC notes that TTO has none of these properties. Therefore, they concluded that classification was not warranted for corrosive to metals.

#### **Classification proposed by the Agency:**

The Agency agrees with RAC's conclusion on classification. Tea tree oil warrants classification as **Flam. Liq 3; H226 (Flammable liquid and vapour)** as per the criteria outlined in Annex I, Table 2.6.1 of GB CLP (e.g. flash point  $\geq 23$  °C and  $\leq 60$  °C).

TTO does not warrant classification for any other physical hazards.

## Health Hazards

### Acute Toxicity

#### Classification agreed by RAC:

##### *Acute toxicity – oral route*

RAC assessed three oral acute toxicity studies. The first study (Anonymous, 2015a) was OECD TG 425 and GLP compliant, and performed using Wistar rats (3/females/group). An LD<sub>50</sub> of 1049 mg/kg bw was established, based on no mortality at 550 mg/kg bw and 100% mortality at 2000 mg/kg bw. The second study (Anonymous, 1989a) was performed according to OECD TG 401 in Sprague Dawley (SD) rats with a specific pathogen free (SPF) and non-SPF group. An LD<sub>50</sub> OF 1682-1721 mg/kg bw was established. The final study (Anonymous, 2010), performed according to OECD TG 423 and GLP in CRL:(NMRI)BR mice (3 females/group) resulted in an LD<sub>50</sub> exceeding 2000 mg/kg bw

In acute oral studies provided by the DS on the constituents of TTO, LD<sub>50</sub>s ranged between 1280 and 4750 mg/kg bw.

Based on the lowest LD<sub>50</sub> of 1049 mg/kg bw obtained in the reliable rat study (Anonymous, 2015a), which fell within the guidance value (GV) range for classification with Category 4 (300 < ATE ≤ 2000 mg/kg bw), RAC concluded that TTO should be classified as Acute Tox. 4; H302 (Harmful if swallowed) with an ATE of 1050 mg/kg bw (rounded up).

##### *Acute toxicity – dermal route*

There were two studies available to assess the acute dermal toxicity of TTO. The first study (Anonymous, 2015b) was performed in line with OECD TG 402 and GLP in Wistar rats. The second study (Anonymous 1989b) was performed according to OECD TG 402 but in New Zealand White (NZW) rabbits.

Both studies reported in LD<sub>50</sub> values of >2000 mg/kg bw.

RAC concluded that classification was not warranted for acute dermal toxicity.

##### *Acute toxicity – inhalation route*

Two studies were available. The first study (Anonymous, 2010a) was performed in Wistar rats (5/sex/dose) according to OECD TG 403 and GLP. The animals were exposed via nose-only inhalation to aerosolised TTO at concentrations of 0, 0.77, 3.69 and 5.06 mg/L.

Mortality was reported in 1 animal at 0.77 mg/L, 4 animals at 3.69 mg/L and 7 at 5.06 mg/L. This gave an LC<sub>50</sub> of 3.64 mg/L.

The second study (Anonymous, 2011) was performed according to OECD TG 403 and GLP in Wistar rats (5/sex/dose). Animals were exposed to TTO via nose-only inhalation at concentrations of 0, 1.94, 3.70 and 5.04 mg/L for 4 hours. LC<sub>50</sub> values were determined to be 5.23 mg/L for males, 4.29 mg/L for females and 4.78 mg/L (combined).

Based on the lowest LC<sub>50</sub> of 3.64 mg/L (Anonymous, 2010a), which fell within the GV range for classification with Category 4 (1.0 < ATE ≤ 5.0 mg/L, dusts and mists), RAC concluded that TTO should be classified as Acute Tox. 4; H332 (Harmful if inhaled), with a rounded down ATE of 3.60 mg/L (dusts and mists).

#### **Classification proposed by the Agency:**

##### *Acute toxicity – oral route*

The Agency agrees with RAC's conclusion on classification. **Classification of TTO as Acute Tox. 4; H302 (Harmful if swallowed), with an ATE of 1050 mg/kg bw, is warranted.**

##### *Acute toxicity – dermal route*

The Agency agrees with RAC's conclusion on classification. **TTO does not warrant classification for acute dermal toxicity.**

##### *Acute toxicity – inhalation route*

The Agency agrees with RAC's conclusion on classification. **Classification of TTO as Acute Tox. 4; H332 (Harmful if inhaled), with an ATE of 3.60 mg/L (dusts and mists), is warranted.**

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

### Classification agreed by RAC:

RAC provided a table summarising the reported clinical signs seen in the available acute toxicity studies (see Table 4).

**Table 4: Effects observed in acute toxicity studies, relevant for STOT SE, taken from pages 9-10 of the RAC opinion (ECHA, 2023)**

Route/ species	Clinical signs	Mortality	LD <sub>50</sub> or LC <sub>50</sub>	Reference
Oral, rat	550 mg/kg bw: - 2000 mg/kg bw: hypoactivity and slight tremors (before dying)	2000 mg/kg bw: N=3 dead 550 mg/kg bw: N=3 survived	1049 mg/kg bw	Anonymous, 2015a
<i>Oral, rat</i>	<i>SPF rats: Surviving animals lack of tonus in forelimbs  Non-SPF rats: 2.15 mL/kg bw: 2 animals had lack of tonus in forelimbs 2.10/1.7 mL/kg bw: all animals had lack of tonus in forelimbs</i>	<i>SPF rats 2.5 mL/kg bw: 3/10 2.6 mL/kg bw: 9/10 2.75 mL/kg bw: 7/10 3 mL/kg bw: 7/10 Non-SPF rats: 1.70 mL/kg bw: 6/10 2.10 mL/kg bw: 3/10 2.15 mL/kg bw: 8/10 2.25 mL/kg bw: 10/10 2.4 mL/kg bw: 10/10</i>	<i>(2.6 in SPF and 1.9 mL/kg bw in non-SPF rats)  1682-1721 mg/kg bw</i>	<i>Anonymous, 1989a</i>
Oral, mice	2000 mg/kg bw: complete lack of muscular tone in forelimbs		>2000 mg/kg bw	Anonymous, 2010
Dermal, rat	No clinical signs		>2000 mg/kg bw	Anonymous, 2015b
Dermal, rabbit	No clinical signs		>2000 mg/kg bw	Anonymous, 1989b
Inhalation, rat	0.77, 3.69, 5.06 mg/L: nasal discharge, slight salivation, lethargy, tremors, ataxia, dyspnoea, perineum wet with urine, dullness, recumbency	Control: 0/10 0.77 mg/L: 1/10 3.69 mg/L: 4/10 5.06 mg/L: 7/10	3.64 mg/L	Anonymous, 2010a
<i>Inhalation, rat</i>	<i>1.94, 3.70 and 5.04 mg/L: wet fur</i>		<i>4.78 mg/L</i>	<i>Anonymous, 2011</i>

*Rows in italics are data present in the DRAR but not in the CLH report.*

RAC also noted various human poisoning cases, relevant for STOT SE, were summarised in the DRAR. These are outlined in Table 5 below:

**Table 5: Human poisoning cases, relevant for STOT SE, taken from page 10 of the RAC opinion (ECHA, 2023)**

Case	Amount of TTO and route	Symptoms	Reference
			All from DRAR, Volume 3-B.6, 2022
4-year-old boy	Ingestion small quantity of TTO (2 teaspoons)	Ataxic, progressing to unresponsiveness, recovered after 24 h	(Morris et al., 2003)
17-month male child	Ingestion of < 10 mL TTO	Ataxia and drowsiness	(del Beccaro, 1995)
23-month male child	Ingestion of < 10 mL	Confused, unable to walk 30 minutes, asymptomatic after 5 h	(Jacobs & Hornfeldt, 1994)
One person	Half a cup of pure TTO (0.5-1 mL/kg bw)	In coma for 12 h, disturbance of consciousness for another 36 h	(Seawright, 1993)
1.6-year female child	10-15 mL TTO oral	Problem with balance, ataxia, confusion, agitation	Zuzak et al. 2010
2-year old child	TTO, amount not specified	Emesis	Zuzak et al. 2010

RAC also considered a publication from the DRAR. Villar *et al.* (1994) described TTO toxicity in dogs and cats, with the main symptoms consisting of depression, weakness, incoordination and muscle tremors. Furthermore, another study highlighted by the European Medicines Agency (EMA, 2014) showed that cats treated with 120mL of undiluted TTO exhibited hypothermia, incoordination, dehydration and trembling. According to RAC, one of these cats died but this was likely due to a pre-existing renal condition. The other two cats recovered 1-2 days following treatment. When considering the reported narcotic effects (e.g. dyspnoea, lethargy, tremors and ataxia) after inhalation exposure in the animal dataset, RAC noted that these effects were reported in one study below the LC<sub>50</sub> (Anonymous, 2010a). For the oral studies, they noted the lack of tonus in the forelimbs was reported without mortality in mice (Anonymous, 2010).

In regards to the human data, RAC noted symptoms of ataxia, drowsiness and coma, and considered these to be evidence of central nervous system depression. Furthermore, as the effects were reversed within 36 hours in all cases, RAC considered them to be transient.

Overall, RAC noted that the effects reported above are examples of narcotic effects which are transient in nature as described in Annex I, Table 3.8.1 of the CLP Regulation and Section 3.8.2.2.2. of the Guidance on the Application of the CLP criteria (ECHA; 2024).

Therefore, they concluded that TTO should be classified for STOT SE 3; H336 (May cause drowsiness or dizziness), for narcotic effects.

#### **Classification proposed by the Agency:**

The Agency disagrees with RAC's conclusion on the classification for STOT SE.

As noted in Section 3.8.2.5 of the ECHA Guidance on the Application of the CLP Criteria (ECHA, 2024), STOT SE and acute toxicity are independent of each other and both may be assigned to a substance if the respective criteria are met. However, care should be taken not to assign each class for the same effect, which would result in double classification. The available animal studies reported effects indicative of narcosis, although the findings were not consistent across all studies. Furthermore, indications of narcosis were generally reported at doses which also caused mortality - the Agency considers that such findings do not warrant classification for STOT SE 3, as they are already captured by the proposed classifications for acute toxicity in this case (where death appears to occur following central nervous system depression).

RAC noted the lack of tonus in the forelimbs of mice at 2000 mg/kg bw, without mortality. However, this dose is above the identified LD<sub>50</sub> (albeit based on rat data), and in the absence of any further information regarding this finding, the Agency does not consider it sufficient to warrant classification. In the additional studies in cats and dogs, the extent of the narcotic effects are not presented (e.g. incidence, severity).

With regards to the human data, a small number of case studies are available where the substance has been accidentally ingested:

1. A 4-year old boy ingested a small quantity (< 10 ml) of TTO and became ataxic and progressed to unresponsiveness (Morris *et al.*, 2003). The child had significant medical interventions (he was “endotracheally intubated by paramedics”), therefore the Agency considers it possible he had ingested a lethal dose. The boy had recovered 24 hours after admission.
2. A 17-month old male child developed ataxia and drowsiness following ingestion of a small quantity (< 10 mL) of TTO (del Beccaro, 1995). This case is difficult to assess, as the exact dose is unknown, as are the medical interventions that the boy received (if any).
3. A 23-month old male child ingested a small quantity (< 10 ml) of T36-C7, a commercial product containing 100% TTO (Jacobs & Hornfeldt, 1994). He became confused and was unable to walk thirty minutes after ingesting. The child was referred to a nearby hospital; it is not clear what treatment they received, but they were asymptomatic after 5 hours.
4. One person lapsed into coma for 12 hours after ingesting half a cup of pure TTO and suffered disturbances of consciousness for another 36 hours. Symptoms of abdominal pain and diarrhoea continued for approximately 6 weeks after this (Seawright, 1993). No information on any medical interventions is available. The Agency considers these effects to be beyond narcosis, and suggest that a (potentially) lethal dose was ingested in this case.
5. A 1.6-year old female child ingested 10-15 ml TTO and developed problems with balance, ataxia, confusion and agitation (Zuzak *et al.*, 2010). No information on any medical interventions is available, making the case difficult to assess.

6. A 2-year old child ingested an unknown amount of TTO, resulting in emesis (Zuzak *et al.*, 2010). In the absence of any further information (on amount ingested, medical interventions, other symptoms, etc.), this report does not provide convincing evidence for classification.

Overall, the human data presented in the DRAR and RAC Opinion (ECHA, 2023) are limited. Only a small number of case reports are available, mostly describing accidental ingestion by young children, where the exact dose is unknown. The Agency has not reviewed the full case reports. Based on the information available in the RAC Opinion (ECHA, 2023) and the DRAR, the Agency considers that the human data do not provide robust evidence of narcotic effects at non-lethal doses.

**Therefore, the Agency concludes that classification for STOT SE is not warranted.**

## **Skin corrosion/irritation**

### **Classification agreed by RAC:**

Three studies were considered in the CLH report, and RAC further considered Table 19<sup>4</sup> of the CLH report, which presented data on human skin patch testing, where irritation was reported.

An OECD TG 404 dermal/irritation study in NZW rabbits (Anonymous, 2015c) was available. A quantity of 0.5mL TTO was applied to the skin of rabbits for 4 hours. Mean erythema scores were 2, 2 and 2.67, and mean oedema scores were 1 for all animals after 24-76 hours.

A non-guideline, non-GLP study (Anonymous, 1989c) was also available, in which undiluted TTO was applied to the skin of six NZW rabbits. Mean erythema and oedema scores were 3.08 and 1.83, respectively, for intact skin, and 3.25 and 2, respectively, for abraded skin.

A Draize skin irritation study (Lee *et al.*, 2013) reported Draize scores of 1 for both oedema and erythema after 24 and 48 hours exposure of Wistar rats to 5% TTO. Scores were 1 and 2 for oedema and erythema, respectively, when the concentration was increased to 10% TTO.

Within the human patch test data, RAC noted a study by Sabroe *et al.* (2016), who highlighted 3 patients out of a total of 2104 with irritant reactions to 5% TTO in petrolatum. Veien *et al.* (2004) reported on weak irritant reactions in 44/217 patients to a 5% TTO

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<sup>4</sup> The RAC opinion cited this table as Table 26 of the CLH report

commercial lotion. The same study group also reported irritant reactions in 5/160 patients to 5% TTO commercial lotion. Skin irritancy was also reported in other human patch tests.

RAC also referred to an opinion by the Scientific Committee on Consumer Products (SCCP, 2008). They noted two skin irritation animal studies and four human studies, not presented in the CLH report. The SCCP concluded that these studies suggested that TTO and 5% formulations of TTO were irritant to the skin. RAC also considered an opinion by the EMA (2014), which reported on the two animal studies referred to by the SCCP, and another study by Halcon & Milkus (2004), which reported a Draize skin irritancy index of 5.0 after application of 100% TTO to intact and abraded skin of albino rats. Human data were also reported by the EMA, who concluded that undiluted TTO causes skin irritation in a small proportion of subjects (<5%). It was postulated that the age of the oil could be the cause of the irritation potential of TTO, since aged oils may contain higher levels of peroxides and degradation products. Finally, RAC referenced the Cosmetic Ingredient Review (CIR, 2011) who summarised various animal and human data, concluding that formulations of 5% TTO or more can induce skin irritation.

Overall, RAC concluded that TTO should be classified as Skin Irrit. 2; H315 (Causes skin irritation) based on erythema/oedema mean values of  $\geq 2.3$  -  $\leq 4.0$  seen 24-72 hours after patch removal, which were reversible. Supporting evidence came from human patch tests.

#### **Classification proposed by the Agency:**

The Agency agrees with RAC's conclusion on classification. **Classification of TTO as Skin Irrit. 2; H315 (Causes skin irritation) is warranted.**

### **Serious eye damage/irritation**

#### **Classification agreed by RAC:**

Three studies were available. The first study discussed by RAC tested 3 male NZW rabbits according to OECD TG 405 and GLP (Anonymous, 2015d). Animals were administered 0.1mL undiluted TTO into the conjunctival sac of the left eye. Mean scores over 24-72 hours were 0 for iris and cornea and 1 for redness, chemosis and discharge in the conjunctiva (1.3 in one rabbit for discharge).

RAC also referred to a study available on the ECHA dissemination site (Anonymous, 2013), in which two male rabbits were tested according to OECD TG 405 and GLP. None of the endpoint mean values (iris, cornea, conjunctival redness/chemosis/discharge) met the criteria for classification.

Lastly, RAC assessed a bovine corneal opacity and permeability (BCOP) study, conducted according to OECD TG 437 and GLP (Anonymous, 2012). *In vitro* irritancy scores were 2.3 for the negative control, 2.2 for TTO and 44.5 for the positive control.

None of the studies produced results that met the criteria for classification in accordance with Tables 3.3.1 or 3.3.2 of Annex I to the CLP Regulation (e.g. corneal opacity  $\geq 1$  and/or iritis  $\geq 1$ , and/or conjunctival redness  $\geq 2$  and/or conjunctival oedema (chemosis)  $\geq 2$ ). Therefore, RAC concluded that classification was not warranted for serious eye damage/irritation.

#### **Classification proposed by the Agency:**

The Agency agrees with RAC's conclusion on classification. **TTO does not warrant classification for serious eye damage/eye irritation.**

### **Respiratory sensitisation**

Not assessed in the CLH report or RAC opinion.

### **Skin sensitisation**

#### **Classification agreed by RAC:**

Two Guinea Pig Maximisation Tests (GPMT) and 4 Local Lymph Node Assays (LLNA) were presented in the CLH report. Furthermore, human patch test data was summarised from the open literature (Table 19 of the CLH report), and several case reports from a review by the Danish Toxicology Centre (Larsen & Borling, 2000) were available for assessment.

The animal data are summarised in Table 6:

**Table 6: *In vivo* skin sensitisation studies, taken from pages 15-16 of the RAC opinion (ECHA, 2023)**

Species, strain, sex, no/group	Test substance	Dose levels / duration of exposure	Results	Reference / reliability score / guideline
<b>Skin Sensitization Study (Magnusson and Kligman) in Guinea Pigs</b>				
Guinea Pig Albino, NIH (Duncan Hartley) males and females 10 controls, 20 in the test item group	Tea tree oil 9.7 % α-Terpinene, 2.6 % 1,8-Cineole, 17.8 % γ-Terpinene, 1.5 % p-Cymene and 41.5 % Terpinen-4-ol  Positive control 2-mercapto benzothiazole	Induction: 25 % (w/w) in propylene glycol Boosting: 50 % (w/w) in acetone Challenge: 100 % TTO (undiluted) Test duration was 48 h	In the control and treatment group, there were no skin reactions at 24 and 48 hours post removal of the test patch. In the positive control group, 6/10 guinea pigs had score of 1 (discrete or patchy erythema) at 24 and 48 hours post removal of the test patch. *	Anonymous, 2015e 1 OECD TG 406 / GLP
Guinea-Pig HA-strain 20 animals per group	Tea tree oil  (no positive control mentioned)	2 weeks after induction, test group challenged by maximum sub-irritant conc (30 % TTO in petroleum jelly) for 24 hours	No dermal responses at challenge (all zero).	Anonymous, 1989d 2 OECD TG 406
<b>LLNA test</b>				
Mouse (CBA/CaHsdRcc (SPF)) Female 5/dose/ group	Melaleuca alternifolia, ext., Purity 100 % Positive control: alpha-hexylcinnamaldehyde in acetone/ olive oil (4/1 v/v)	2 %, 20 % in PEG 300 and 100 % negative control group with PEG 300	Stimulation index (SI) (Mean): 2.4 at 2 % (SD=1.4) 6.9 at 20 % (SD=2.0) 16 at 100 % (SD=6.3) EC3=4.4 % (w/v) Positive control results provided in study 2006a, below Slight ear erythema observed at all doses; scales on ears in high dose	ECHA dissemination site; Anonymous, 2006 1 OECD TG 429 / GLP
Mouse (CBA/CaHsdRcc (SPF)) Female 5/dose/ group	Melaleuca alternifolia, ext., Purity 100 % Positive control: alpha-hexylcinnamaldehyde in acetone/ olive oil (4/1 v/v)	2 %, 20 % in PEG 300 and 100 % negative control group PEG 300	SI (Mean): 1.6 at 2 % (SD=0.4) 2.8 at 20 % (SD=0.7) 5.7 at 100 % (SD=1.6) EC3=25.5 % (w/v) Positive control results: SI (Mean): 1.8 at 5 % SI (Mean): 2.9 at 10% SI (Mean): 6.2 at 25% EC3=10.5 % (w/v) No erythema or scales on ears of all mice	ECHA dissemination site; Anonymous, 2006a 1 OECD TG 429 / GLP
Mouse (CBA/CaHsdRcc (SPF)) Female 5/dose/group	Melaleuca alternifolia, ext., Purity 100 % Positive control: alpha-hexylcinnamaldehyde in acetone/ olive oil (4/1 v/v)	2 %, 20 % in PEG 300 and 100 % negative control group with PEG 300	SI (Mean): 1.8 at 2 % (SD=0.4) 2.8 at 20 % (SD=1.2) 6.5 at 100 % (SD=2.3) EC3=24.3 % (w/v) Positive control results provided in study 2006a, above Slight ear erythema in two highest dose groups	ECHA dissemination site; Anonymous, 2006b 1 OECD TG 429 / GLP
Mouse (CBA/J) Female 5/dose/group	Melaleuca alternifolia, ext., Purity 100 % Positive control: alpha-hexylcinnamaldehyde 25 % in PEG 400	5 %, 25 % and 50 % in PEG 400 negative control group with PEG 400	SI (Mean): 2.1 at 5 % (SD=0.7) 7.7 at 25 % (SD=4.0) 7.9 at 50 % (SD=3.2) EC3=8.3 % (w/v) Positive control results: SI (Mean): 21.2 at 25 % (SD=7.7) No dermal irritation found at all doses	ECHA dissemination site; Anonymous, 2007 2 Method similar to OECD TG 429 / GLP

\*data as presented in the DRAR.

RAC noted that the 4 LLNAs were positive and of acceptable reliability. They noted that the 2 negative GPMTs contradicted these results, but did not consider the results of the GPMTs to overrule the results of the LLNAs. RAC also highlighted that the positive control data for both GPMTs was poor or non-existent (e.g. only 60% of guinea pigs responded to the positive control with discrete or patchy erythema in the Anonymous (2015e) test, and no positive control was reported in the Anonymous (1989d) study). They considered this to reduce the sensitivity of these tests.

The human data also confirmed that TTO is a skin sensitiser. Sabroe *et al.* (2016) tested 5% TTO in 2104 patients. Eleven patients were positive, 2 doubtful and 3 were reported as irritant. Veien *et al* (2004) tested 10% TTO in petrolatum and 5% TTO commercial lotions in 217 patients, and reported 65 positive patch tests (30%). In another test by the same authors, four commercial lotions containing 5% TTO were applied to 160 patients. No allergic reactions were reported, but 5 patients did show irritancy. RAC concluded that Category 1B classification seems more appropriate based on the human data than Category 1A, since incidences were low and concentrations were high.

Additionally, RAC acknowledged a review by the Danish Toxicology centre (Larsen & Borling, 2000), which reported that oxidised TTO is a stronger sensitiser than fresh TTO. RAC noted that in the LLNA studies, TTO was stable (e.g. not oxidised) and only caused irritation in 2/4 studies. They further noted that irritation is also reported in the human patch tests. Section 3.4.2.2.2 of the Application of the CLP guidance (ECHA, 2024) notes that when a substance auto-oxidises to a more hazardous form, the parent compound may still warrant classification.

Finally, RAC made reference to limonene and  $\alpha$ -Terpinene, highlighting that both of these components of TTO are classified as skin sensitisers<sup>5</sup>.

RAC considered that, overall, the available data indicated that TTO is a skin sensitiser. Therefore, they concluded that TTO warranted classification as Skin Sens. 1B; H317 (May cause an allergic skin reaction).

#### **Classification proposed by the Agency:**

The Agency agrees with RAC's conclusion on classification. **TTO warrants classification as Skin Sens. 1B; H317 (May cause an allergic skin reaction).**

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<sup>5</sup> Limonene (CAS: 138-86-3) and  $\alpha$ -Terpinene (CAS: 99-86-5) are both listed in Annex VI of EU CLP and on the GB MCL list with a classification of Skin Sens. 1; H317 (May cause an allergic skin reaction).

## Specific target organ toxicity – repeated exposure (STOT RE)

### Classification agreed by RAC:

Several repeated dose toxicity studies were included for assessment, with additional information coming from reproductive toxicity studies.

No effects were reported in a 28d study in SD rats dosed via oral gavage with up to 45 mg TTO/kg bw/d (Anonymous, 2017b). However, in a second 28d study (Anonymous 2010b), where Wistar rats (6/sex/dose) were dosed via oral gavage with 0, 62.5, 125 and 250 mg TTO/kg bw/d, male animals showed reduced testes and epididymis weights from 125 mg/kg bw/d. The weight reductions were statistically significant at 250 mg/kg bw/d (mean absolute/relative decreases of 34/28 and 43/39% in testes and epididymis weights, respectively, compared to the control group). These weight changes were seen alongside degeneration in the testes (6 and 4 animals at 250 and 125 mg/kg bw/d, respectively), and oligospermia (1 and 2 animals at 250 and 125 mg/kg bw/d, respectively) and cell debris (4 animals at 125 mg/kg bw/d) in the epididymis. These effects were not observed in any control animals. Statistically significant increases in liver weights were seen in females from 125 mg/kg bw/d (absolute/relative increases of 19/18% at 125 mg/kg bw/d and 23/32% at 250 mg/kg bw/d) and males (relative increase of 14%) at 250 mg/kg bw/d, only. Relative adrenal weights were also increased by 18% at the top dose for both sexes.

Three 90d studies were available. Two of these were conducted to OECD TG 408 and GLP (Anonymous 2011b and 2016a) in Wistar rats (males and females). In the Anonymous 2011b study, 10 rats/sex/dose were administered TTO via oral gavage at dose levels of 0, 30, 60 and 120 mg/kg bw. Concurrent recovery groups for the vehicle control and the top dose consisted of 20/animals/sex. Effects were reported in the sperm (significant reduction in counts and motility, along with significant increases in the percentage of abnormal sperm) from 60 mg/kg bw/d, and at the top dose (120 mg/kg bw/d), there were histopathological changes in the testes (degeneration in seminiferous tubules and Sertoli cell vacuolation in 8 and 9 animals, respectively) and epididymis (sperm granuloma in 4 animals). These effects were not observed in the control group. Minimal degrees of spleen vacuolation (5 animals) and dilation of tubules in the kidneys (3 animals) were also reported at the top dose. In the 2016a study, sperm counts and sperm motility were reduced, and degeneration/atrophy of the seminiferous tubules were reported at the only dose of 60 mg/kg bw/d. One 90d study was conducted according to OECD TG 409 and GLP (Anonymous 2018a) in Beagle dogs. Intoxication was reported at the two highest doses (180 and 75 mg/kg bw/d), and these were subsequently reduced to 120 and 60 mg/kg bw/d, respectively. From 60mg/kg bw/d, the viability and motility of spermatids were statistically significantly decreased; the percentages of live sperm were 54.8% and 63.1% at 75/60 mg/kg bw/d and 180/120 mg/kg bw/d, respectively, compared to

83.4/16.6% in the control group, and immotility was 76.0% and 55.6% at 75/60 mg/kg bw/d and 180/120 mg/kg bw/d, respectively, compared to 29.3% in the control group.

A two-generation reproductive toxicity study (Anonymous, 2017a) and two prenatal developmental toxicity studies were also discussed in the CLH report (Anonymous, 2012a and Anonymous, 2018b). No effects relevant for STOT RE were observed.

In the CLH report, the DS considered that the spermatogenesis effects may be relevant for STOT RE. However, they concluded that gavage was not a relevant route of administration so did not consider this further. However, RAC did not concur with the DS. They supported the use of oral gavage for the purposes of classification. Nonetheless, the effects on the testes, epididymis and sperm are more relevant for classification in reproductive toxicity, hence this was further discussed by RAC in this section.

RAC also considered the effects seen in the liver/adrenals. Minimal vacuolation of the liver, although seen in some studies, was not considered to be significant enough for classification. Furthermore, RAC noted that the liver weights were increased in the 90d studies from 60 mg/kg bw/d but without any corresponding histopathological correlates.

RAC concluded that **classification was not warranted for TTO for STOT RE.**

#### **Classification proposed by the Agency:**

The Agency agrees with RAC's conclusion on classification. **TTO does not warrant classification for STOT RE.**

### **Germ cell mutagenicity**

#### **Classification agreed by RAC:**

Eight *in vitro* studies and one *in vivo* study were available for the assessment of germ cell mutagenicity. These are summarised in the table below:

**Table 7: Summary of germ cell mutagenicity data, taken from pages 18-19 of the RAC opinion (ECHA, 2024)**

Parameter	Concentration	Results	Reference/ reliability score
<b><i>In vitro</i> studies</b>			
Bacterial Reverse Mutation Test OECD TG 471 GLP	TA 98, TA 100, TA 1535 and TA 1537 strains of <i>Salmonella typhimurium</i> and WP2 uvrA (pKM 101) strain of <i>Escherichia coli</i> .	Negative	Anonymous, 2010b 1
Bacterial Reverse Mutation Test	TA98, TA100 and TA102 strains of <i>Salmonella typhimurium</i> .	Negative	ECHA dissemination site; Anonymous, 1989 2
Mammalian cell gene mutation test OECD TG 476 GLP	Mouse lymphoma L5178Y cells	Negative, with and without metabolic activation	ECHA dissemination site; Anonymous, 2010 1
Mammalian Cell Gene Mutation Test OECD TG 476 GLP	Chinese hamster Ovary cells (CHO)	Negative, with and without metabolic activation	Anonymous, 2015f 1
<i>In vitro</i> mammalian chromosomal aberration test OECD TG 473 GLP	Chinese hamster lung fibroblasts (V79)	Negative, tested up to cytotoxic concentrations, with and without metabolic activation	ECHA dissemination site; Anonymous, 2009 1
<i>In vitro</i> mammalian micronucleus test Similar to OECD TG 487	Human lymphocyte cultures	Negative	Pereira et al., 2014 2
<i>In vitro</i> mammalian chromosomal aberration test Similar to OECD TG 473	Human lymphocyte cultures	Negative	Pereira et al., 2014 2
<b><i>In vivo</i> studies</b>			
Mouse Micronucleus Test	Oral administration of 1000 (10 % w/w), 1350 and 1750 mg TTO/kg bw 4 groups of 5 males and 5 females	Negative	Anonymous, 2005 1

All of the available *in vitro* studies were negative with/without metabolic activation, and were considered to demonstrate no potential to induce DNA or chromosomal damage. The single *in vivo* micronucleus study was also negative, and bone marrow exposure was considered to be proven, since a decrease in polychromatic erythrocytes to total erythrocytes was observed. Additional literature studies, provided in the CLH report, were negative also.

RAC concluded that no classification was warranted for germ cell mutagenicity.

#### **Classification proposed by the Agency:**

The Agency agrees with RAC's conclusion on classification. **TTO does not warrant classification for germ cell mutagenicity.**

## Carcinogenicity

### Classification agreed by RAC:

There were no studies available to assess TTO carcinogenicity.

Within the CLH report, the following was noted by the DS with regards to the assessment of TTO (text taken from page 20 of the RAC opinion):

#### Reproduced ECHA text:

- TTO has a long history of safe use in a wide range of products.
- Consumers are not exposed, due to lack of residues on treated crops.
- TTO/its components are metabolised and rapidly cleared within 2-3 days, so no potential for bioaccumulation. In addition, TTO components are metabolised into non-hazardous metabolites.
- Small fraction of TTO remains in the body, so unlikely to cause any long-term effects such as carcinogenicity.
- TTO was tested negative in genotoxicity studies.
- Several studies demonstrate that TTO and its main component terpine-4-ol have anti-carcinogenic activities, both in vitro and in vivo.
- High volatility (DRAR).
- Natural occurrence of TTO and its components in the environment (DRAR)

#### End of reproduced ECHA text

The CLH report also provided additional information on TTO's components and carcinogenicity (text taken from page 20 of the RAC opinion):

#### Reproduced ECHA text:

- A carcinogenicity study was performed in female mice with intraperitoneal injections of 1900 or 9600 mg/kg bw of  $\alpha$ -terpineol or  $\beta$ -terpineol, 3 times a week for a total of 24 doses. After 24 weeks no dose related tumours were found.
- A carcinogenicity study (Bhowal & Gopal, 2015) was performed with toothpaste ingredients, including eucalyptol (1,8-cineole) in male SPF CFLP mice (n=52 per group) at a dose of 8 or 32 mg/kg bw/d by gavage, 6 days per week for 80 weeks. No notable differences in the incidence or severity of tumours.
- In a primary lung tumour model (A/HE), 12 g eucalyptol/kg bw intermittent was tested, but resulted to be negative for tumour induction (Bhowal & Gopal, 2015).

- D-limonene was tested by oral gavage in mice and rats with known carcinogens as cancer-preventive agent (Jameson, 1990; IARC, 1999). It was shown to inhibit lung carcinogenesis in mice, preneoplastic stages of colon carcinogenesis in rats, and pancreatic carcinogenesis in hamsters.
- D-limonene exposure results in renal tumours in male rats only, caused by an  $\alpha$ 2u-globulin-associated response.

#### **End of reproduced ECHA text**

The CLH report suggested that since there are carcinogenicity studies in 1,8-cineole, terpineol and limonene (>95% of TTO components), which do not report carcinogenic effects, it is unlikely that TTO would be carcinogenic.

RAC noted that arguments related to safe use, exposure, biotransformation, rapid clearance and lack of bioaccumulation are not relevant arguments to dismiss the carcinogenic potential of TTO. The evidence from components of TTO were considered to provide some evidence, but RAC ultimately noted that this is not enough to dismiss a carcinogenic potential for TTO as a whole.

Therefore, RAC concluded that classification was not warranted for carcinogenicity, based on lack of data.

#### **Classification proposed by the Agency:**

The Agency agrees with RAC's conclusion on classification. **TTO does not warrant classification for carcinogenicity.**

### **Reproductive toxicity**

#### **Classification agreed by RAC:**

A two-generation reproductive toxicity study and three prenatal developmental toxicity (PNDT) studies (two in rats and one in rabbits) were available for assessment.

#### Sexual Function and Fertility

A two-generation reproductive toxicity study (Anonymous, 2017a) was performed according to OECD TG 416 and GLP. Wistar rats were exposed by oral gavage to 0, 10, 25 and 50 mg TTO/kg bw/d for the parental generation, whilst the F1 generation was exposed to 0, 10, 25 and 38 mg/kg bw/d. The top dose was reduced in the F1 generation owing to alterations in reproductive performance. Furthermore, there were only 23 F1 females in the top dose compared to 25 in all other groups.

A dose-related decrease in the number of pregnancies was reported (92, 84, 84 and 56% in the control, 10, 25 and 50 mg/kg bw/d, respectively) in the parental generation. The fertility index was also significantly lower in both sexes at the top dose (in males, 44% compared to 84% in the control, and in females, 56% compared to 92% in the control), and this was associated with decreased sperm motility (12% reduction in motile sperm) and cauda epididymal sperm counts (32%), plus a 486% increase in abnormal sperm counts. Statistically significant decreases in the mean number of corpora lutea and implantations were also reported at the top dose (counts were 9.3 and 6.7, respectively, compared to 12.8 and 11.1 in the control), with a significantly higher number of pre implantation losses (33.4% compared to 15.0% in the control). The mean litter size was 10, 8.7, 9 and 7 for the control, 10, 25 and 50 mg/kg bw/d groups, respectively. The F1 generation had a small reduction in the number of pregnancies at the top dose (87% vs 100% in controls). Cauda sperm counts were also lower at this dose (19% reduction in the number of sperms per cauda epididymis and 18% reduction in the number of sperms per gram of cauda epididymis).

The CLH report also noted adverse effects on the testes and/or sperm count/motility in repeated dose toxicity studies (28-day study and two 90-day studies in rats and a 90-day study in dogs). RAC summarised these findings in the table below:

**Table 8: Summary of studies showing adverse effects on male fertility, taken from page 26 of the RAC opinion (ECHA, 2024)**

Study	Testes weight	Sperm count	Motility	Abnormal sperm	Histopathology
28-days rat (Anonymous, 2010b)	↓ at 250 mg/kg bw/d	-	-	-	Degenerative changes in testes (minimal to mild)
90 days rat (Anonymous, 2011b)	no change	↓ at 60 /120 mg/kg bw/d	↓ at 60 /120 mg/kg bw/d	↓ at 60 /120 mg/kg bw/d	Degenerative changes in testes, Sertoli cell vacuolation at 120 mg/kg bw/d
90-days rat (Anonymous, 2016a)	-	↓ at 60 mg/kg bw/d	↓ at 60 mg/kg bw/d	↓ at 60 mg/kg bw/d	Some slight changes
90-days dog (Anonymous, 2018a)	-	-	↓ at 75/60 mg/kg bw/d	-	-

↑ or ↓ refer to a statistically significant change.

- No data

In conclusion, RAC considered the effects on testes, epididymides, sperm counts and motility across the two generation study and repeated dose toxicity studies to be sufficient for classification with Category 1B (H360F). They also noted that female fertility could also be affected, since the number of corpora lutea and implantations were lower and pre-implantation loss was higher.

RAC did consider arguments against a classification of Category 1B:

- It was noted by stakeholders that relevance to humans is doubtful, since no effects were reported in humans exposed to high components of TTO in food. Both RAC and the DS confirmed that no human data on TTO exist. During the public consultation, the DS provided their thoughts on the expected exposure of humans to TTO, in a response to a comment submitted by the Agency. RAC considered that information on exposure was not relevant for hazard assessment. All comments related to low/no exposure in humans were dismissed on the grounds that exposure is not considered for hazard assessment.
- A mode of action (MoA) was also proposed by commenters during the public consultation. These suggested that effects on spermatogenesis occur due to a constituent of TTO (p-cymene). It was noted that p-cymene is metabolised to p-isopropyl benzoic acid (p-iPBA) which is then conjugated to co-enzyme A. This conjugation can accumulate in the testes and at a certain threshold lead to errors in lipid metabolism which may lead to the apical effects. The commentor highlighted that there is a quantitative species difference in metabolite accumulation between humans and rats. However, RAC noted that P-cymene only makes up 0.5-8% of TTO and that this makes it scientifically impossible to exclude another MoA.
- It was suggested by the DS that studies using oral gavage may be irrelevant for human exposure. RAC disagreed with this argument, and noted that studies using oral gavage can be used for hazard classification.
- Commenters also referred to long history of safe use of monocyclic terpenes in diet and other products. However, with no relevant epidemiological studies in humans, these arguments could not be supported.

Overall, RAC concluded that TTO should be classified as Repr. 1B; H360F (May damage fertility).

### Developmental Toxicity

A PNDDT study (Anonymous, 2012a) was conducted according to OECD TG 414 and GLP. Wistar rats (24/dose) were administered 0, 75, 150 and 300 mg TTO/kg bw/d via oral gavage from gestation day (GD) 5-8, and this was reduced to 0, 30, 60 and 120 mg/kg bw/d from GD 8-19 owing to severe clinical signs and mortality. A statistically significant reduction in maternal body weight gain and food consumption was reported in the 150/60 and 300/120 mg/kg bw/d groups (e.g. corrected body weight gain was +18.24 g, +9.33 g and -4.40 g for control, 150/60 and 200/120 mg/kg bw/d, respectively). There was an increased number of resorptions seen in the 150/60 and 300/120 mg/kg bw/d groups (47.8% and 57.1%, respectively, compared to 25% in controls). At the same two doses, mean foetal weight was also reduced by 4.6% and 15%, respectively. Ossification delay was also seen in various bones at the same doses. RAC considered that the effects seen in this study were likely to be due to maternal toxicity and therefore not relevant for classification.

In another OECD TG 414 and GLP compliant PNDT (Anonymous 2011), Wistar rats (up to 27/group) were exposed to 0, 20, 100 or 250 mg TTO/kg bw/d via oral gavage from GD 5-19. Seven animals at the high dose died, and maternal body weight was reduced by 20 and 45% at doses of 100 and 250 mg/kg bw/d, respectively. Foetal body weight was also reduced at both of these doses (by 13% and 32%, respectively). Increased incidences of external and skeletal malformations were also seen in the foetuses of the high dose group (e.g. displaced rib cartilages at the sternum, malformed vertebrae, and/or short bent scapula, humerus or femur). Again, RAC considered that the effects in this study were likely a result of maternal toxicity, as foetal effects were seen only at high doses where mortality and reduced body weight were reported.

In a final PNDT study (Anonymous, 2018b) conducted according to OECD TG 414 and GLP, NZW rabbits (24/group) were exposed orally by gavage to 0, 15, 30 or 75 mg/kg bw/d from GD 6-28. In this study, no maternal toxicity was seen. There was a statistically significant increase in the number of post-implantation losses in the high dose (1.76 vs 0.52 in control). The DS attributed this finding to the statistically significant decrease in food consumption in the dams at 30 and 75 mg/kg bw/d (96.82 and 88.85g/rabbit/d, respectively, compared to 133.75g/rabbit/d in the control). However, RAC noted that the maternal body weight was not significantly affected, and the corrected body weight gain was not statistically significantly reduced. Furthermore, decreased food consumption and post implantation loss failed to show a relationship. RAC cited the CLP criteria (Annex I; section 3.7.2.4.2), which states that *“Developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity. Moreover, classification shall be considered where there is a significant toxic effect in the offspring, e.g., irreversible effects such as structural malformations, embryo/foetal lethality, significant post-natal functional deficiencies”*. Since these effects cannot be unequivocally attributed to maternal toxicity, RAC considered the post-implantation loss in rabbits in their final conclusions.

Overall, RAC considered the post-implantation loss in rabbits, which was statistically significant at the top dose (mean incidence) and showed a dose related response, to be relevant for classification. Effects reported in the rat studies (PNDT and two generation study discussed under sexual function and fertility) were considered to be of more questionable relevance, as they coincided with maternal toxicity. Therefore, RAC concluded that TTO should be classified as Repr. 2 (H361d).

#### Lactation

RAC noted that there was no data available on the presence of TTO components in breast milk.

Within the two-generation reproductive toxicity study (Anonymous 2017a), no effects on post-natal survival, lactation or viability indices in either generation were reported following TTO exposure. There was a reduction in the mean body weight of the F2 generation on post-natal day (PND) 1 and 4 (male pups) and PND 1, 4 and 7 (female pups & combined sexes) at the highest dose of 38 mg/kg bw/d. Similar findings were not seen in the F1 generation, even up to a higher dose of 50 mg/kg bw/d. At the end of lactation (21 days), body weights were recovered and in line with the controls. Nonetheless, RAC noted that even if the reduction in body weights were treatment-related, they should not be considered as a severe toxic effect. They further noted that nursing behaviour was not impaired.

RAC concluded that no classification was warranted for effects on or via lactation.

### **Classification proposed by the Agency:**

#### Sexual function and fertility

The Agency agrees with RAC's conclusion on classification. **TTO should be classified for Repr. 1B; H360F (May damage fertility).**

#### Developmental Toxicity

RAC considered that the dose-related increase in post implantation loss seen within the PNDT study in NZW rabbits (Anonymous 2018b) is sufficient to classify TTO as Repr. 2 for developmental toxicity.

However the Agency disagrees that this is sufficient to warrant classification. The post implantation loss in this study was 0.52, 0.65, 0.76 and 1.76\* for the control, 15, 30 and 75 mg/kg bw/d groups, respectively. RAC included the historical control data (HCD) in their numerical summary table (page 27-28 of the RAC opinion; ECHA (2024)), which states that in 222 NZW rabbits, a mean post implantation loss of 0.45 with a standard deviation of 0.78 and range of 0-4 was reported. The statistically significant value at the top dose was within the range of the HCD and had a standard deviation of  $\pm 1.84$ , which suggests that there was a lot of variation within this dose group. During the targeted public consultation, a company-downstream user (comment 11) highlighted that one female in the high dose group had 100% resorption, which they suggested to cause the higher mean value in the top dose. RAC responded saying that multiple dams had several resorptions that contributed to the higher mean post-implantation loss. They provided a table outlining the individual post implantation losses of each dam from every exposure group; Table 9 below. It does suggest a slightly higher number of dams with any resorptions but the difference between the dam with 100% resorptions and the dam with second highest absorptions (57.1%) is considerable. The dam with the second highest resorption rate is also more in line with the other dose groups and control which all have at least one dam with 50% resorptions.

**Table 9: Individual data on % post implantation loss in the 2018b study, taken from page 10 of the targeted consultation RCOM (ECHA, 2024)**

Control group	15 mg/kg bw/day	30 mg/kg bw/day	75 mg/kg bw/day
-	14.3	12.5	100.0
-	-	50.0	18.2
-	11.1	-	42.9
-	-	-	16.7
10.0	14.3	28.6	-
-	-	66.7	25.0
-	-	33.3	16.7
-	12.5	12.5	-
20.0	33.3	-	22.2
-	33.3	-	57.1
12.5	-	-	-
37.5	-	14.3	22.2
12.5	-	-	16.7
-	-	12.5	-
-	-	-	30.0
-	12.5	25.0	-
-	-	-	-
-	14.3	-	20.0
-	14.3	-	-
-	-	-	25.0
-	33.3	-	42.9
12.5	-	11.1	-
50.0	-	-	50.0
14.3	50.0	11.1	20.0

RAC also reported that this study showed no maternal toxicity which could unequivocally explain the increased post-implantation loss. However, a dose-dependent reduction in corrected maternal body weight was seen (-200g at the top dose compared to -3.6g in control) which, although not statistically significant, is in line with the statistically significant reduction in food consumption seen from a dose of 30 mg/kg bw/d. At the top dose, food consumption was reduced by ~34% compared to controls.

Therefore, considering that the increased post-implantation loss was only slightly increased at the top dose (possibly due to an outlier), and coincides with considerably reduced food consumption the Agency considers this finding insufficient to support classification for developmental toxicity.

Therefore, the Agency considers that **TTO does not warrant classification TTO for adverse effects on development.**

## Lactation

During the CLH consultation, the Agency requested the numerical data on pup body weight loss, so that a firm conclusion could be established. The DS provided this in the RCOM. Taking this into consideration alongside the conclusion of the RAC opinion, the Agency agrees that the reduction in pup body weight seen in the F2 generation would not warrant classification.

Overall, the Agency agrees with RAC's conclusion on classification. **Classification is not warranted for effects on or via lactation.**

## **Aspiration hazard**

### **Classification agreed by RAC:**

Since the viscosity of TTO (containing >10% hydrocarbons) is 1.71 mm<sup>2</sup>/s at 40°C, which is below 20.5 mm<sup>2</sup>/s as specified by the classification criteria in the CLP Regulation, RAC concluded that classification for Asp. Tox. 1; H304 (May be fatal if swallowed and enters airways) is warranted.

### **Classification proposed by the Agency:**

The Agency agrees with RAC's conclusion on classification. **Classification of TTO as Asp. Tox. 1; H304 (May be fatal if swallowed and enters airways) is warranted.**

## **Environmental hazards**

### **Hazardous to the aquatic environment**

Where environmental fate or ecotoxicity data have been used in the classification, the material tested was considered by the DS and RAC to be broadly representative of the substance subject to this classification. Certain sections of the classification assessment are also based on data available on the individual components of TTO, in particular the highest % w/w ones like terpinen-4-ol. A summary of current EU harmonised and self-classifications for the 15 components of TTO is given in Table 59, Section 2.11.1.2 of the original CLH Report.

## Classification agreed by RAC:

### Rapid degradability of organic substances

In their Opinion (ECHA, 2023) RAC referred to the ECHA Guidance on the Application of the CLP Criteria (ECHA, 2017) for detailed recommendations on how the results of biodegradability tests for complex substances, such as UVCBs, should be carefully evaluated before their use in hazard classification. RAC also provided a tabular summary of the relevant physical-chemical properties of individual TTO constituents, such as vapour pressure, volatility, Henry's Law Constant and the adsorption coefficient, log  $K_{oc}$ . Overall, RAC determined that TTO was **not rapidly degradable** for the purpose of hazard classification based on the following information presented in their Opinion. This was in contrast to the DS who had considered the substance to be rapidly degradable in the CLH Report (ECHA, 2022):

- RAC cited a section (4.1.3.2.2 d) of the ECHA Guidance on the Application of the CLP Criteria which states that '*Biodegradation, bioaccumulation, partitioning behaviour and water solubility all present problems of interpretation, where each component of these complex or multi-constituent substances may behave differently*'. Also Annex II.3.1 to the Guidance, which states that rapid degradability requires '*a more detailed assessment of the degradability of the individual constituents in the complex substance. When the constituents that are not-rapidly degradable constitute a significant part of the complex substance, e.g. more than 20 %, or for a hazardous constituent, an even lower content, the substance should be regarded as not rapidly degradable*'. From the available physical-chemical data RAC considered that all five constituent monocyclic monoterpenes, aliphatic and aromatic hydrocarbons, as well as the three bicyclic monoterpene constituents, have a vapour pressure value above 50 Pa at 25°C. Also taking the extreme low water solubility of three polycyclic sesquiterpenes in account, RAC considered that 11 out of the 15 known constituents of TTO have a high Henry's law constant and volatility.
- In addition, RAC noted that only two of the 15 known constituents of TTO have a low Log  $K_{oc}$  value (< 3), whilst four constituents have a high Log  $K_{oc}$  value above 3.0, or a very high Log  $K_{oc}$  value above 5.0. This indicated a high risk of dissipation from biodegradation testing via adsorption or formation of non-extractable residues (NER). RAC noted that nine of the 15 known constituents have no reported Log  $K_{oc}$  value.
- As a consequence of these observations, RAC noted that some constituents of TTO are difficult to study in biodegradation test systems and any result from a biodegradation test needed to be evaluated with high scrutiny to ensure that it truly indicate rapid biodegradation and that the calculated DT<sub>50</sub> value represented only a DegT<sub>50</sub> and is not influenced by rapid dissipation, e.g. by volatilisation, adsorption and/or formation of NER - which would cause the test to be invalid and unreliable.

On the basis of this information, RAC determined that the degradability of TTO needed to be assessed separately for each constituent (as far as possible) as well as for the whole substance.

- In terms of abiotic degradation, RAC noted that no hydrolysis study (or information on photodegradation in water) was available, either for the whole UVCB TTO or for the separate constituents.
- The DS had relied on a ready biodegradation study on TTO performed to GLP and OECD TG 310 (Fiebig, 2010, cited in ECHA, 2022) which indicated the whole substance to be readily biodegradable with 87 % biodegradation observed by day 7. The 10 % degradation level was reached after 2 days and the 60 % pass level was reached within the 10-day time window, after 5 days. The maximum biodegradation came to 106 % after 28 days.
- RAC noted that this TG 310 ready biodegradation study on TTO was based on CO<sub>2</sub> production in sealed vessels (a headspace test) and that the OECD Guidelines for the Testing of Chemicals - revised Section 3 Introduction, Part 1 (OECD, 2006) indicated that ready biodegradability tests are intended for pure substances and are generally not applicable for complex compositions containing different types of constituents, like UVCBs. Furthermore, the test report only gave details for five of the 15 known constituents - which sum up to approximately 77 % of the TTO test material. The remaining 23 % of constituents were not known and it was unclear if the total carbon content (the source of the measured CO<sub>2</sub>) was fully accounted for.
- RAC also noted point 11 in OECD TG 310 which says '*Using the recommended headspace to liquid volume ratio of 1:2, volatile substances with a Henry's law constant of up to 50 (Pa x m<sup>3</sup>)/mol can be tested as the proportion of test substance in the headspace will not exceed 1 %. A smaller headspace volume may be used when testing substances, which are more volatile, but their bioavailability may be limiting especially if they are poorly soluble in water.*' Despite the high Henry's law constant (> 50 Pa x m<sup>3</sup>/mol) of the majority of constituents, the head space was not adjusted and normal headspace flasks with 120 mL were used.
- RAC concluded that the above uncertainties and shortcomings relating to the OECD TG 310 test on TTO were sufficient to question the reliability of the results and make them difficult to interpret.
- A 2018 study had been performed according to GLP and OECD TG 308 (aerobic transformation in two water/sediment systems) on key components of TTO (terpinene-4-ol,  $\gamma$ -terpinene, p-cymene, 1,8-cineole, aromadendrene and globulol). This study indicated apparent rapid degradation with reported DT<sub>50</sub> values for some of the components of < 16 hours, and with no degradants > 10% AR identified in the water or sediment phases over the whole study period. However, terpinene-4-ol (max. 48 % w/w in TTO) was the only radiolabelled compound used in the study; this showed whole system DT<sub>50</sub> values of 104 to 196 hours and formation of degradants was not determined for the other main TTO constituents. Potential losses to volatilisation were also not measured and the derived FOCUS kinetics

were considered by the DS to not be fully reliable or representative of actual degradation. Although there was rapid dissipation of some components from the water phase, complete whole system mineralisation for all components was not well reported. RAC concluded, in line with the DS, that the results obtained from this TG 308 study were not sufficiently reliable to describe degradation. The DT<sub>50</sub> values should be regarded as dissipation rather than solely degradation half-lives, since most of the tested constituents have a high Henry's law constant and some have a high log K<sub>oc</sub>, so they would be expected to partition to the air and sediment phases. An available TG 307 (aerobic transformation in soil) study was also considered of limited value to describe degradation in aquatic systems.

- Focussing on ready biodegradation of the individual constituents of TTO, RAC noted that the aerobic biodegradation of  $\delta$ -cadinene (max. 3% w/w presence in TTO) had been measured in an OECD TG 301F Manometric Respirometry test (Jenner *et al.*, 2011, cited in ECHA, 2022). Removal of ThOD was < 60 % after 28 days and it was concluded that this constituent of TTO was not readily biodegradable. The purity of  $\delta$ -cadinene in the test material was only 63.2 % though, with other sesquiterpenes also present, and so the results may be questionable. RAC also noted however that Finland had conducted an EU REACH Substance Evaluation on resin and rosin acids, hydrogenated, esters with glycerol (CAS No 65997-13-9). In the SEv conclusion indications of potential persistence for some sesquiterpenes (including  $\delta$ -cadinene) were presented.
- During written consultation between RAC Members, further evidence became available on other single constituents of TTO which had not been taken into account by the DS. RAC noted that some constituents have their own REACH registration dossier and are considered not readily biodegradable by the Registrants themselves. For example, RAC noted that in a publicly available biodegradation study (OECD TG 301 F) for  $\gamma$ -terpinene (max. 28 % w/w in TTO) only 27 % degradation was observed after 28 days, leading to the Registrant for  $\gamma$ -terpinene concluding that it is not readily biodegradable. Due to the high vapour pressure of  $\gamma$ -terpinene, the reliability of this test result might be questioned however, and it was not assessed by RAC.
- In its Opinion of 2019 on  $\alpha$ -terpinene (max. 13% w/w in TTO), RAC also concluded that this component substance was not rapidly degradable on the basis of data from an OECD TG 301F test (which showed 40% biodegradation after 28 days).
- RAC assessed all 15 constituents of TTO using their SMILES (Simplified Molecular Input Line Entry System) code with the BioWin v4.10 software - and none of the results indicated the individual components to be readily biodegradable. However, the reliability and the QSAR applicability domains were not evaluated by RAC, therefore these predicted results were only taken into account as supporting information.

In conclusion, on the basis of the above information, RAC considered TTO to be not rapidly degradable for the purpose of environmental hazard classification since, according to Section 4.1.3.2.3.2 of the Guidance on the Application of the CLP Criteria '*a substance is considered to be not rapidly degradable unless the rapid degradability has been proven*'. No reliable data were available to allow RAC to conclude on rapid degradability of the whole substance, whereas information was available which indicated 'non-rapid degradability' for at least three constituents representing up to 44 % w/w of TTO. Referring to Annex II.3.1 of the Guidance on the Application of the CLP Criteria (see first bullet above) - where constituents that are not-rapidly degradable constitute a significant part of the complex substance, e.g. more than 20 %, the whole substance should be regarded as not rapidly degradable.

### Bioaccumulation

RAC agreed that TTO had the potential to be **bioaccumulative** for the purpose of hazard classification based on the following information presented in their Opinion (ECHA, 2023). This was in contrast to the DS who had considered the substance to be not bioaccumulative in the CLH Report (ECHA, 2022):

- RAC identified that for UVCB substances like TTO, the potential for bioaccumulation needed to be assessed separately for each constituent and cannot be measured in one experimental BCF test for the whole UVCB (ref. Guidance on the Application of the CLP Criteria - Annex III.3.2). Measured BCF values were not available for any of the known constituents of TTO. RAC noted that experimentally measured Log K<sub>ow</sub> values were available for many of the constituents and these are usually considered more reliable than estimated BCFs to determine bioaccumulation potential.
- The measured and estimated Log K<sub>ow</sub> values of the known constituents of TTO were tabulated in the RAC Opinion. For 12 of the 15 components the Log K<sub>ow</sub> was greater than the CLP trigger value of  $\geq 4$ , and six of these 12 also had an estimated BCF value (taken from the CLH Report) above the CLP trigger of 500; only three of the 15 known constituents had an experimentally measured log K<sub>ow</sub> below the trigger of 4 and an estimated BCF below the trigger of 500.

In conclusion, based on the above information on a large proportion of the constituents, and in the absence of more reliable experimental BCF data, RAC determined that the whole UVCB substance TTO should be considered potentially bioaccumulative for the purpose of environmental hazard classification.

### Aquatic Toxicity

In their opinion (ECHA, 2023), RAC considered that reliable short- and long-term aquatic toxicity data for TTO were available in the CLH Report (ECHA, 2022) for fish,

invertebrates, algae and aquatic plants. In addition, reliable regulatory and literature studies were available on some of the individual components of TTO.

Acute toxicity:

Aquatic invertebrates were the most acutely sensitive trophic group with the lowest short-term **48-hour EC<sub>50</sub> of 0.591 mg/L** for *Daphnia magna* (95% confidence limits: 0.499 - 0.700 mg/L) using TTO. This semi-static 2011 study was conducted to OECD TG 202 and GLP with endpoints based on geometric mean measured concentrations. The DS considered this study to be Reliability 1 noting validity criteria were met, although it is not clear from CLH Report what particular component(s) of TTO was analysed for or what percentage of nominals was obtained. Acute aquatic endpoints for other taxa, and acute studies on some of the individual TTO components, were not discussed in the RAC Opinion but are available in the CLH Report.

RAC considered the *D. magna* study to be reliable, and as the EC<sub>50</sub> fell within the range  $0.1 < L(E)C_{50} \leq 1$  mg/L, they concluded that TTO should be classified as **Aquatic Acute 1 (H400) with an M-factor of 1**.

Chronic toxicity:

RAC considered that fish were the most chronically sensitive trophic group, with the lowest long-term **34-day NOEC of 0.244 mg/L** from an early life stage (ELS) test with *Pimephales promelas* (fathead minnow) (95% confidence limits: 0.499 - 0.700 mg/L). This flow-through 2017 study was conducted to OECD TG 210 and to GLP and the endpoint was based on cumulative survival over 34 days (28 days post-hatch) and mean measured concentrations. The DS considered this study to be Reliability 1, although it is again unclear from the CLH Report which component(s) of TTO was analysed. Chronic aquatic endpoints for other taxa were not discussed in the RAC Opinion but are available in the CLH Report, there were no long-term studies presented on individual TTO components.

In the CLH report, the DS proposed to classify TTO as Aquatic Chronic Category 3 based on the above chronic fish NOEC but considering TTO to be 'rapidly degradable'. Given RAC considered TTO to be 'not rapidly degradable' and the chronic fish NOEC fell in the range  $0.1 < NOEC/EC_{10} \leq 1$  mg/L, RAC agreed TTO should be classified as **Aquatic Chronic Category 2 (H411)**.

#### RAC Opinion

Overall, RAC agreed to classify Tea Tree Oil (TTO) as:

- **Aquatic Acute 1 (H400) with an M-factor of 1** based on the 48-hour EC<sub>50</sub> of 0.591 mg/L for *Daphnia magna*.
- **Aquatic Chronic 2 (H411)** based on the 34-day early life stage NOEC of 0.244 mg/L for

*Pimephales promelas* and for a not rapidly degradable / potentially bioaccumulative substance.

### **Classification proposed by the Agency:**

The Agency agrees that TTO can be considered **not rapidly degradable** for the purpose of hazard classification. Given the reassessment of the available OECD TG 310 'headspace' ready biodegradation study by RAC and their investigation of key physical-chemical properties of individual components of TTO, the Agency agrees that the interpretation and reliability of this test is called in to question. This is mainly because, as identified by RAC, a significant proportion of the components show a high potential to dissipate to air or adsorb to organic material and surfaces within the test system. The study had not been adapted appropriately to account for this (e.g. reduced headspace) and the total carbon content of the test system also does not appear to have been fully accounted for. With 10 of the 15 known constituents no conclusion can be drawn based on results of this test.

Similarly, the Agency agrees with RAC that the OECD TG 308 water/sediment simulation study on main components of TTO did not fully take in account potential losses to volatilisation and adsorption and the quoted DT<sub>50</sub> values may not be fully reliable or representative of actual degradation. There was limited evidence relating to the formation of degradants and complete mineralisation - and the DT<sub>50</sub> values obtained probably relate more to dissipation rather than degradation.

The Agency agrees with the various sections of the ECHA and OECD guidance quoted by RAC which identify that rather than relying on a single degradability test on a whole complex UVCB substance, information on the individual components should be taken in to account. In this case there is sufficient information identified by RAC to conclude that a significant number of the components of TTO (> 20 % w/w in maximum total) are not rapidly degradable and so the Agency agrees that the whole TTO substance cannot therefore be considered rapidly degradable. The constituents of TTO have a wide range of physical-chemical properties and TTO is not a homologous series of substances within a certain range of carbon chain length and/or degree of substitution which further demonstrates that the single degradability test on the whole UVCB substance should not be relied upon.

The Agency agrees that TTO is potentially **bioaccumulative** for the purpose of hazard classification. No experimental BCF test data are available on the complete UVCB or on any of its components. A range of measured and estimated Log K<sub>ow</sub> values and estimated BCF values are presented in the CLH Report and RAC Opinion. The Agency agrees that the measured Log K<sub>ow</sub> values provide the most reliable indication of overall bioaccumulation potential and that this should be assessed separately for each

constituent. The Log K<sub>ow</sub> was greater than the CLP criterion of  $\geq 4$  for 12 of the 15 components of TTO and, in total, the possible maximum % w/w of those components with log K<sub>ow</sub> values  $\geq 4$  exceeds 50% of the UVCB. Therefore, in the absence of experimental BCF data, the Agency agrees that the whole TTO substance should be considered potentially bioaccumulative under CLP.

The Agency agrees that reliable short-term toxicity data on fish, invertebrates and algae/aquatic plants are available for acute hazard classification. These mostly relate to the whole TTO substance but some data are also available on individual components of the UVCB ( $\alpha$ -terpineol, p-cymene and  $\alpha$ -pinene). For all of the acute studies on fish, algae and plants (*Lemna gibba*), the L/EC<sub>50</sub> values were  $> 1$  mg/L for TTO and these components. The Agency agrees that the most acutely sensitive endpoint is the **48-hour EC<sub>50</sub> of 0.591 mg/L** for *Daphnia magna* derived from a GLP OECD TG 202 study. The endpoint is described as geometric mean measured but it is not clear what particular component, or components, of TTO were analysed for or what percentage of nominals was achieved. Given the physical-chemical properties of many of the TTO components, there could have been volatilisation and adsorption of test material during this test and no measures to adapt the test system to minimise this are reported. The study was however performed under semi-static conditions and it is presumed there would have been sufficient short-term exposure. The data available on the toxicity of p-cymene and  $\alpha$ -pinene to *D. magna* indicate these individual components to be substantially less acutely toxic (E/LC<sub>50</sub> values of 6.5 and 41 mg/L respectively), the EC<sub>50</sub> for TTO as a whole therefore remains the lowest acute endpoint.

As the TTO EC<sub>50</sub> for *D. magna* falls within the range  $0.1 < L(E)C_{50} \leq 1$  mg/L, the Agency agrees that TTO should be classified as **Aquatic Acute 1; H400 (Very toxic to aquatic life) with an Acute M-factor of 1**.

The Agency agrees that reliable long-term toxicity data are also available for all trophic groups. The available data all relate to the whole UVCB TTO and no long-term data were presented in the CLH Report or RAC Opinion on individual components of TTO. The Agency agrees that the lowest reliable chronic endpoint is the **34-day NOEC of 0.244 mg/L** for *Pimephales promelas* obtained from a GLP OECD TG 210 early life stage test. An EC<sub>10</sub> was not determined. The endpoint relates to cumulative survival and was based mean measured concentrations although it was also not clear which components of TTO were analysed. A number of the components of TTO have high volatility and low solubility and so maintaining concentrations of these may have been difficult. Reflecting this, measured concentrations in the test media during the exposure period were reported to be in the range of 5% to 49% of nominals. Given the study employed a flow-through design, exposure to TTO is at likely to have been reasonably consistent.

Whilst algae and *Lemna* were less chronically sensitive to TTO (NOE<sub>rC</sub> values  $> 1$  mg/L), a 21-day 'measured' NOEC of 0.303 mg/L based on adult mortality and EC<sub>10</sub> of 0.411

mg/L based on reproduction, were reported from a GLP OECD 211 study with *Daphnia magna* (the most acutely sensitive species). Whilst this has similar uncertainties to the chronic fish test regarding analytics, both studies were considered Reliability 1 by the DS and these endpoints are in the same hazard classification range and support the chronic classification.

As the long-term fish NOEC of 0.244 mg/L falls within the range  $0.1 < \text{NOEC}/\text{EC}_{10} \leq 1$  mg/L and TTO as a UVCB substance is considered to be not rapidly degradable (and potentially bioaccumulative) the Agency agrees that TTO should be classified as **Aquatic Chronic Category 2; H411 (Toxic to aquatic life with long lasting effects)**.

## Other hazards

### Hazardous to the ozone layer

Not assessed in the CLH report or RAC opinion.

## Overall conclusion

The Agency has evaluated the RAC Opinion, its rationale and any additional scientific evidence that may have been made available to HSE against the criteria for classification and labelling in the GB CLP Regulation and technical guidance.

The Agency technical report **agrees** with the classification proposed by RAC for the following hazards:

Flam. Liq. 3; H226 (Flammable liquid and vapour)

Acute Tox. 4; H302 (Harmful if swallowed)

Acute Tox. 4; H332 (Harmful if inhaled)

Skin. Irrit. 2; H315 (Causes skin irritation)

Skin Sens. 1B; H317 (May cause an allergic skin reaction)

Repr. 1B; H360F (May damage fertility)

Asp. Tox. 1 (H304 – May be fatal if swallowed and enters airways).

The Agency technical report **disagrees** with the classification proposed by RAC for the following hazards:

STOT SE 3; H336 (May cause drowsiness or dizziness). The Agency considers that classification for STOT SE is not warranted.

Repr. 2; H361d (Suspected of damaging the unborn child). The Agency considers that classification for developmental toxicity is not warranted.

Overall, the conclusion is to **disagree** with the RAC opinion.

## References

ECHA (2017) Guidance on the Application of the CLP criteria. Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures, version 5.0, ref: ECHA-17-G-21-EN. Available at <https://www.echa.europa.eu/>

ECHA (2024) Guidance on the Application of the CLP criteria. Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures, version 6.0, ref: ECHA-24-G-01-EN. Available at <https://www.echa.europa.eu/>

OECD (2006) Revised Introduction to the OECD Guidelines for Testing of Chemicals, Section 3, Available at: <https://www.oecd-ilibrary.org/>. Accessed date: 07/2024

**For all other references, please see the EU CLH report and the EU RAC opinion (available at: <https://echa.europa.eu/registry-of-clh-intentions-until-outcome>)**

CLH (2022) CLH report (including Annexes): Proposal for Harmonised Classification and Labelling Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2. Substance Name: Melaleuca alternifolia, ext. [1] Melaleuca alternifolia, essential oil; tea tree oil [2]; Date: 2022; Written by: Poland Accessed date: 02/2024

ECHA (2023) Committee for Risk Assessment (RAC) Opinion (including Annexes) proposing harmonised classification and labelling at EU level of Melaleuca alternifolia, ext. [1] Melaleuca alternifolia, essential oil; tea tree oil [2]; Reference CLH-O-0000007380-79-01/F; Date: 30/11/2023, Accessed date: 02/2024

**Documents published as part of the EU CLH process: Source: European Chemicals Agency, <http://echa.europa.eu/>**

## Glossary of terms used in Agency technical reports

<b>Agency, the</b>	HSE, acting in its capacity as the GB CLP Agency
<b>AR</b>	Applied radioactivity
<b>ATE</b>	Acute toxicity estimate
<b>BCF</b>	Bioconcentration factor
<b>BCOP</b>	Bovine corneal opacity and permeability
<b>BOD</b>	Biological Oxygen Demand
<b>bw</b>	Body weight
<b>CAR</b>	Competent Authority Report
<b>CAS</b>	Chemical Abstracts Service
<b>CI</b>	Confidence interval
<b>CL</b>	Confidence limits
<b>CLH</b>	Harmonised Classification and Labelling
<b>CLP</b>	Classification, labelling and packaging (of substances and mixtures)
<b>CO<sub>2</sub></b>	Carbon dioxide
<b>COD</b>	Chemical Oxygen Demand
<b>CIR</b>	Cosmetic Ingredient Review
<b>CV</b>	Coefficient of Variation
<b>D</b>	Day
<b>DAR</b>	Draft Assessment Report
<b>DOC</b>	Dissolved Organic Carbon
<b>DRAR</b>	Draft Renewal Assessment Report
<b>DS</b>	Dossier Submitter
<b>DSC</b>	Differential Scanning Calorimetry
<b>DT</b>	Dissipation time OR degradation time (also DissT or DegT where apparent)
<b>DT<sub>50</sub></b>	Dissipation half-life OR degradation half-life (hours or days), see also above
<b>dw</b>	Dry weight
<b>ECHA</b>	European Chemicals Agency
<b>EC<sub>x</sub></b>	x% effect concentration
<b>EFSA</b>	European Food Safety Authority
<b>EMA</b>	European Medicines Agency
<b>E<sub>r</sub>C<sub>x</sub></b>	x% effect concentration based on growth rate
<b>EU</b>	European Union
<b>GD</b>	Gestation Day
<b>GLP</b>	Good Laboratory Practice

<b>GPMT</b>	Guinea Pig Maximisation Test
<b>GV</b>	Guidance Value
<b>H</b>	Hours
<b>HCD</b>	Historical Control Data
<b>K<sub>oc</sub></b>	Organic carbon-water partition coefficient
<b>K<sub>ow</sub></b>	Octanol-water partition coefficient
<b>LC<sub>x</sub></b>	x% lethal effect concentration
<b>LLNA</b>	Local Lymph Node Assay
<b>MCL</b>	Mandatory Classification and Labelling
<b>M-factor</b>	Multiplying factor
<b>MoA</b>	Mode of Action
<b>MW</b>	Molecular weight
<b>NOEC</b>	No-observed effect concentration
<b>NZW</b>	New Zealand White
<b>OECD</b>	Organisation for Economic Co-operation and Development
<b>p-IPBA</b>	p-isopropyl benzoic acid
<b>PNDT</b>	Prenatal developmental toxicity study
<b>PND</b>	Post Natal Day
<b>QSAR</b>	Quantitative structure-activity relationship
<b>RAC</b>	Risk Assessment Committee
<b>RAR</b>	Renewal Assessment Report
<b>RCOM</b>	Response to comments document
<b>REACH</b>	Registration, Evaluation, Authorisation and Restriction of Chemicals regulation
<b>SCCP</b>	Scientific Committee on Consumer Products
<b>SD</b>	Sprague Dawley
<b>SMILES</b>	Simplified Molecular Input Line Entry System
<b>SPF</b>	Specific Pathogen Free
<b>STOT-RE</b>	Specific target organ toxicity – repeated exposure
<b>STOT-SE</b>	Specific target organ toxicity – single exposure
<b>TG</b>	Test Guideline
<b>TTO</b>	Tea Tree Oil
<b>UN RTDG</b>	United Nations Recommendations for the Transport of Dangerous Goods
<b>US EPA</b>	United States Environmental Protection Agency
<b>UVCB</b>	Unknown or variable composition, complex reaction products or biological materials
<b>wt</b>	Weight
<b>wwt</b>	Wet weight









## Further information

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